CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Elsevier - Publisher Connector

Available online at www.sciencedirect.com
ScienceDirect
Virology 372 (2008) 85–96
VIROLOGY
www.elsevier.com/locate/yviro

Molecular characterization of L-413C, a P2-related plague diagnostic bacteriophage

Emilio Garcia [a,*,1], Patrick Chain [a,b,1], Jeff M. Elliott [a], Alexander G. Bobrov [c,2], Vladimir L. Motin [a,3], Olga Kirillina [c,2], Victoria Lao [a], Richard Calendar [d], Andrey A. Filippov [c,4]

[a] *Chemistry, Materials and Life Sciences Directorates Lawrence Livermore National Laboratory, Livermore, CA 94550, USA*
[b] *Microbial Genome Program, Joint Genome Institute, Walnut Creek, CA 94598, USA*
[c] *Russian Research Anti-Plague Institute "Microbe", Saratov 410005, Russia*
[d] *Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA*

Received 20 July 2007; returned to author for revision 28 August 2007; accepted 26 October 2007

Abstract

Our analysis of the plague diagnostic phage L-413C genome sequence and structure reveals that L-413C is highly similar and collinear with enterobacteriophage P2, though important differences were found. Of special interest was the mosaic nature of the tail fiber protein H in L-413C, given the differentiating specificity of this phage for *Yersinia pestis* vs. *Yersinia pseudotuberculosis*. While the N-terminal 207 and C-terminal 137 amino acids of L-413C display significant homology with the P2 H protein, a large (465 amino acid) middle section appears to be derived from a T4-related H protein, with highest similarity to the T6 and RB32 distal tail fibers. This finding along with appropriate preadsorption experiments suggest that the unique H protein of L-413C may be responsible for the specificity of this phage for *Y. pestis*, and that the *Y. pestis* receptors that are recognized and bound by L-413C either do not exist in *Y. pseudotuberculosis* or have a different structure.
© 2007 Elsevier Inc. All rights reserved.

Keywords: *Yersinia pestis*; Plague diagnostic bacteriophage; Genome sequencing; Tail fiber; H protein; Preadsorbtion test; Host-specificity

Introduction

Yersinia pestis is the causative agent of plague, one of the most severe human bacterial infections. This disease may have been responsible for taking as many as 200 million lives throughout the history of mankind. Even today, the World Health Organization receives at least 2000 plague case reports annually. *Y. pestis* is also important as it has been and continues to be a potential agent for biowarfare and bioterrorism. Thus, increasing our understanding of virulence and our ability to rapidly detect or identify *Y. pestis* remains an important area of research (Anisimov et al., 2004; Gage and Kosoy, 2005).

Lysis by specific bacteriophages remains an essential method for *Y. pestis* identification and plague diagnosis (Chu, 2000; Dennis et al., 1999; Domaradskij, 1998). *Y. pestis* phages differ in their antigenic properties, morphology of their particles, virulence, genome structure, and level of specificity to the plague bacillus. Immunologically, they have been placed into four serovars. The most common and numerous of them belong to serovar 1, represented by all known lytic plague diagnostic phages (Arutyunov, 1970; Kudryakova et al., 1999; Shashaev, 1964), and serovar 2 which includes L-413C and the majority of *Y. pestis* temperate phages (Larina et al., 1970; Leshkovich et al., 1975; Novoseltsev, 1967; Plotnikov et al., 1982). Serovar 3 to this day consists of only one temperate phage, denominated P (Novoseltsev and Marchenkov, 1990), while serovar 4 is represented by two additional temperate *Y. pestis* phages, Tal and 513 (Novoseltsev et al., 1994).

* Corresponding author. Chemistry, Materials and Life Sciences Directorate, Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550. Fax: +1 925 422 2282.
E-mail address: garcia12@llnl.gov (E. Garcia).
[1] The two first authors contributed equally.
[2] Present address: Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky, Lexington, KY 40536, USA.
[3] Present address: Departments of Pathology and Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555, USA.
[4] Present address: Division of Bacterial and Rickettsial Diseases, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA.

0042-6822/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2007.10.032

Serovar 1 comprises at least 33 *Y. pestis* lytic bacteriophages that have been studied in the former Soviet Union (Arutyunov, 1970; Kudryakova et al., 1999; Shashaev, 1964) including the phages discovered by d'Herelle (d'Herelle, 1926) and by Pokrovskaya (1929), as well as phages φA1122 (Advier, 1933; Garcia et al., 2003), H (Molnar and Lawton, 1969), Y (Hertman, 1964), and 21 other phages described by Smith and Burrows (1962). These all have isometric hexagonal heads and a short (13–14 up to 42 nm) noncontractile conical tail (Arutyunov, 1970; Knapp and Zwillenberg, 1964; Novoseltsev et al., 1971; Plotnikov et al., 1985; Popov and Kirillina, 1982). Thus, they belong to the family Podoviridae, morphotype C1, which include the common phages T3, T7, and φII of *Escherichia coli* as well as P22 of *Salmonella enterica* (Ackermann, 2003). The serovar 1 phages usually show partial plague specificity, lysing 74–100% of *Y. pestis* strains, some isolates of *Yersinia pseudotuberculosis* (25–65%), *Yersinia enterocolitica* (up to 15%), *E. coli*, *Shigella*, and *Salmonella* (Arutyunov and Novoseltsev, 1970; Knapp, 1962; Kudryakova et al., 1999; Smith and Burrows, 1962). Even the Pokrovskaya phage, the best plague diagnostic phage of this serovar currently in use in Russia and other countries from the Commonwealth of Independent States, has been shown to lyse 98–100% of *Y. pestis* cultures and 19–27% of *Y. pseudotuberculosis* isolates in studies that involved thousands of strains (Imamaliev et al., 1986; Larina et al., 1981). For this reason, use of the Pokrovskaya phage has been recommended as an additional test of *Y. pestis* strains only in those rare cases in which lysis with the main diagnostic phage L-413C fails (Imamaliev et al., 1986; Larina et al., 1994).

The φA1122 phage appears to be quite specific and efficient when used at 20 °C for lysing *Y. pestis* but not *Y. pseudotuberculosis* in studies involving a large number of strains (Cavanaugh and Quan, 1953; Garcia et al., 2003; Gunnison et al., 1951). In fact, only two *Y. pestis* strains are known to be resistant to this phage among the thousands deposited in the CDC culture collection (May C. Chu, personal communication). However, this number would likely be much larger if diagnostic studies were extended to include *Y. pestis* isolates from plague foci of the former Soviet Union and Mongolia, which include a large number of atypical (Aparin and Golubinskii, 1989) and some naturally phage resistant strains (Larina et al., 1994; Lebedeva, 2000; Pak et al., 1985). Moreover, *Y. pestis* mutants resistant to serovar 1 diagnostic plague phages can be easily obtained in the laboratory (Kutyrev et al., 1987; Lebedeva, 2000; Pak et al., 1985), hence the use of alternate, more specific diagnostic phages has been sought.

Members of serovar 2 include 17 temperate bacteriophages isolated from lysogenic *Y. pestis* strains, from infected laboratory animals, or from the soils in and around rodent holes of natural plague foci (Larina et al., 1970; Larina and Kishenevskii, 1973; Leshkovich et al., 1975; Novoseltsev, 1967; Plotnikov et al., 1982). Of this group, the phages N (Novoseltsev, 1967) and L-413 (Larina et al., 1970) are the best characterized. These phages display different particle structures possessing an isometric polygonal head of similar size to those of serovar 1 (53–63 nm), but their tail is rather long (110–160 nm) and features a sheath capable of contracting (Larina and Konnov, 1980; Leshkovich et al., 1975; Novoseltsev et al., 1971; Plotnikov et al., 1985). They have genomes that consist of 31–33 kb linear double-stranded DNA (Bobrov et al., 1999; Novoseltsev et al., 1994) and belong to the family Myoviridae, morphotype A1, together with the common enterobacteriophages P1, P2, and P4 (Ackermann, 2003). The phages of serovar 2 can propagate in a wide range of *Y. pestis* strains of various geographical origins and display a rather weak specificity (Larina, 1976; Novoseltsev, 1967; Plotnikov et al., 1982) with the exception of L-413 and L-94. Their lytic mutant derivatives, L-413C and L-94C, lyse only *Y. pestis* and neither *Y. pseudotuberculosis* nor the great majority of *E. coli* strains (Larina, 1976; Larina et al., 1970). Phage L-413 was isolated from the lysogenic virulent *Y. pestis* strain 413 (biovar Medievalis), which was obtained in 1964 from a red-tailed gerbil in a Central Asian Desert plague focus. The clear plaque producing L-413C was selected after multiple passages in the *Y. pestis* host strain (Larina et al., 1970). Its lytic activity spectra compared with the Pokrovskaya phage suggested that L-413C is much more specific for *Y. pestis* as it lysed all *Y. pestis* strains tested but was inactive on other *Yersinia* species and the majority of other enteric bacteria (Larina, 1976; Larina et al., 1970, 1981). For example, in one study, of 103 *E. coli* strains tested, only one, C-85, which is a derivative of *E. coli* C (Bertani and Bertani, 1970), was susceptible to L-413C (Larina, 1976). Further large-scale inter-center testing performed in the FSU, with ca. 7000 strains of *Y. pestis* (isolated in various plague foci all over the world) and more than 1200 strains of *Y. pseudotuberculosis* of five serovars (Imamaliev et al., 1986), confirmed that within members of Yersinia, L-413C has a unique specificity for *Y. pestis* and is a powerful tool for discrimination between *Y. pestis* and its closest relative, *Y. pseudotuberculosis*. By using standard procedures, L-413C has been shown to lyse 99.9% of *Y. pestis* strains and not a single *Y. pseudotuberculosis* isolate. There are only 10 atypical *Y. pestis* strains that are resistant to L-413C (Imamaliev et al., 1986). The few accounts of its ability to lyse strains of *Y. pseudotuberculosis* or inability to lyse rare strains of *Y. pestis* (Imamaliev et al., 1986; Larina et al., 1970, 1981, 1994; Pak et al., 1985) have been shown to be associated with failure to use standard procedures of lysis (Imamaliev et al., 1986; Larina et al., 1994), contamination of tested cultures with soil bacteria (Larina et al., 1994), erroneous discrimination between *Y. pestis* and *Y. pseudotuberculosis* species (Imamaliev et al., 1986; Larina et al., 1981), or existence of rare *Y. pestis* strains carrying phages homoimmune to L-413C (Larina et al., 1994; Pak et al., 1985).

In light of the great practical importance of L-413C, we have obtained its genome sequence and describe here the detailed molecular characterization of this phage focusing on those unique features that confer to this phage its high specificity.

Results

Preliminary L-413C characterization

Growth characteristics of bacteriophage stock on Y. pestis strains

We showed that the initial preparation of the P2-like plague diagnostic bacteriophage L-413C consisted of a mixed

population of both lytic and temperate particles. Determination of titers using *Y. pestis* EV76 (biovar Orientalis, see Table 1) showed that this preparation produced two types of plaques, clear and turbid at titers of 2×10^5 and 4×10^4 pfu/ml, respectively. Lytic phage titers were obtained on EV76 at 28 and 37 °C and found to be 1.2×10^9 and 2×10^7 pfu/ml, respectively. When another host strain *Y. pestis* PKR133 (biovar Medievalis, like strain 413, the original source of L-413) was used, the titer rose one order of magnitude and reached 1.5×10^{10}. Selection of a phage clone from a single large clear plaque with a few subsequent passages on *Y. pestis* PKR133 resulted in a greater L-413C yield, which correlated with highest bacterial lysis, and was observed after 8 h of incubation at 28 °C. All subsequent studies were conducted with purified bacteriophage preparations of this lytic clone.

Table 2
Efficiencies of L-413C and P2 *vir1* plaque formation on *Y. pestis*, *Y. pseudotuberculosis*, and *E. coli* at different temperatures

Phage	L-413C		P2 *vir1*	
Temperature	25 °C	37 °C	25 °C	37 °C
Bacteria	Efficiencies of plating			
E. coli C-1a	1	1	1	1
E. coli C600	NT[a]	2×10^{-4}	NT	0.06
E. coli 802 [C600 $r_k^- m_k^+$]	NT	0.19	NT	0.65
E. coli MG1655	NT	3×10^{-4}	NT	0.12
Y. pestis EV766	0.32	0.26	$<10^{-3}$	0.02
Y. pseudotuberculosis PB1	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
Y. pseudotuberculosis PB1 *ΔddhD-wzz*[b]	2×10^{-5}	2×10^{-5}	0.6	0.4

[a] NT, not tested.
[b] O-antigen negative rough LPS mutant of PB1.

Host specificity of L-413C and P2

We first performed lysis spot tests with L-413C and P2 phages using *Y. pestis* strains KIM D27, CO92, and EV766, as well as three restriction-deficient *E. coli* strains, C-1a, 802, and DH5α (Table 1). In order to ascertain if the surface features of *Y. pestis* grown at 37 °C (e.g. lipopolysaccharide structure changes, production of capsular antigen) affect lysis by L-413C, cells were grown at 28 °C and 37 °C, prior to phage infection. Both *Y. pestis* and *E. coli* strains were susceptible to L-413C at either temperature and surprisingly, P2 also lysed *Y. pestis* strains at 37 °C, but not at 28 °C. The relative efficiencies of L-413C and P2 *vir1* (Bertani, 1957) at making plaques on *E. coli*, *Y. pestis*, and *Y. pseudotuberculosis* were obtained at 25 °C and 37 °C (using bacteria pregrown at the respective temperatures) and are shown in Table 2. Both phages had the highest efficiency of plaquing on *E. coli* C-1a. They also grew on *E. coli* K-12 derivatives but with relatively poor efficiencies of plaquing (especially L-413C) on the strains with intact restriction and modification systems (C600 and MG1655). L-413C showed a better plaque formation on *Y. pestis* than P2, especially at 28 °C. Both L-413C and P2 produced no plaques on PB1, the wild-type, smooth LPS strain of *Y. pseudotuberculosis.* Only P2 plated efficiently on the isogenic rough mutant, PB1 *ΔddhD-wzz* lacking O-antigen (Kiljunen et al., 2005).

Sequence analysis of the L-413C genome

General features

The L-413C bacteriophage genome consists of 30,728 bp, has a G+C content of 52.1%, and encodes 39 predicted

Table 1
The bacterial strains used in this work

Strain	Relevant characteristics	Reference
Y. pestis KIM D27[a]	Pgm⁻; biovar Medievalis	Brubaker (1969)
Y. pestis PKR133	Pgm⁻, pFra⁻ pLcr⁻ pPst⁻; biovar Medievalis	Kutyrev et al. (1989)
Y. pestis CO92 Pgm⁻ Lcr⁻[a]	Pgm⁻ pLcr⁻; biovar Orientalis	Andrews et al. (1996)
Y. pestis EV766[b]	Pgm⁻ pLcr⁻; biovar Orientalis	Portnoy and Falkow (1981)
Y. pestis KM218	Pgm⁻, pFra⁻ pLcr⁻ pPst⁻; biovar Orientalis	Protsenko et al. (1992)
Y. pseudotuberculosis PB1[c]	Wild-type smooth LPS strain, serovar 1b	Burrows and Bacon (1960)
Y. pseudotuberculosis PB1 *Δwb*[c]	The *ΔddhD-wzz* mutation results in loss of O-antigen (rough phenotype)	Kiljunen et al. (2005)
E. coli C-1a[d]	"Restrictionless" P2 phage indicator strain	Sasaki and Bertani (1965)
E. coli C-117[d]	C-1a lysogenized with P2 (immunity group I)	Bertani (1964)
E. coli C-119[d]	C-1a lysogenized with P2 Hy *dis* (group II)	Mandel and Berg (1968)
E. coli C-1917[e]	C-1a lysogenized with φD145 (group VI)	Bertani and Bertani (1971)
E. coli C-1920[d]	C-1a lysogenized with Wφ (group III)	Glover and Kerszman (1967)
E. coli C-1928[e]	C-1a lysogenized with φD160 (group V)	Bertani and Bertani (1971)
E. coli TD204[e]	C-1a lysogenized with HK109 (group IV)	Dhillon et al. (1980)
E. coli E573[f]	K-12 derivative lysogenized with 186 phage (out-group)	J.B. Egan, unpublished
E. coli ECOR4[e]	A natural isolate carrying a defective P2-like prophage (group VII)	Ochman and Selander (1984)
E. coli MG1655	K-12 derivative; λ⁻ *ilvG rfb-50 rph-1* ($r_k^+ m_k^+$)	Bachmann (1996)
E. coli C600[d]	K-12 derivative; λ⁻*thr-1 leu-6 thi-1 supE44 lacY1 tonA21* ($r_k^+ m_k^+$)	Appleyard (1954)
E. coli 802[g]	C600 $r_k^- m_k^+$	Wood (1966)
E. coli DH5α[a]	K-12 derivative; φ80*lacZΔM15* Δ(*lacZYA-argF*) *supE44 thi-1 recA1 gyrA96 endA1 relA1 hsdR17*($r_k^- m_k^+$)	Hanahan (1985)

The strains were kindly provided by [a]M.P. Nikolich (Walter Reed Army Institute of Research); [b]D.A. Portnoy (University of California, Berkeley, CA); [c]M. Skurnik (University of Helsinki, Finland) and B.J. Hinnebusch (Rocky Mountain Laboratories, NIAID, Hamilton, MT); [d]G. Bertani (California Institute of Technology, Pasadena, CA); [e]E. Haggård-Ljungquist (University of Stockholm, Stockholm, Sweden); [f] J.B. Egan (University of Adelaide, Adelaide, Australia); and [g]P. Berg (Stanford University Medical Center, Stanford, CA). All *Y. pestis* strains used were avirulent due to loss of the chromosomal pigmentation region and/or pLcr plasmid.

genes (Table 3). The L-413C genome displays marked homology to the 33,593-bp temperate enterobacteriophage P2 genome (GenBank accession no. AF063097) and to the 32,684-bp P2-like heteroimmune phage Wφ (accession No. AY135739). The genomes display complete colinearity throughout their homologous regions and L-413C also shares the identical terminal 19-bp direct repeats (GGCG-AGGCGGGGAAAGCAC) of both of these phages. The gene organization of L-413C and P2 is displayed in Fig. 1, along with corresponding proteins and their similarity to P2 that are shown in Table 3.

Proteins identical to known phage proteins

Of the 39 coding sequences (CDSs) encoded by L-413C, 11 have 100% identity to sequenced homologs in GenBank, including four (O, K, W, Orf78) to proteins in bacteriophage P2 and one (P) in Wφ. Interestingly, four other CDSs (N, Orf20, C, Cox) are identical to products found in various P2-like phages, isolated from *E. coli* or found in the ECOR collection (Dhillon et al., 1980; Ochman and Selander, 1984), yet are not in P2 itself. Three of these lie within the variable regions of P2 (see "L-413C differences in nonessential, variable regions" section below). Finally, two other CDSs (Y, Orf83) have identical orthologs

Table 3
Gene and gene products of the bacteriophage L-413C genome

Gene name	Product name	Genome position [a]	Codons/amino acids	Function	% P2 identity	P2 CDS (size)	Comments
Q	Q	c189–1223	344	Presumed portal protein	99	344	
P	P	c1223–2995	590	Terminase; DNA-dependent ATPase	99	590	
O	O	3169–4023	284	Presumed capsid scaffolding protein	100	284	
N	N	4082–5155	357	Major capsid precursor	99	357	
M	M	5159–5902	247	Terminase	97	247	
L	L	6002–6511	169	Capsid completion protein	98	169	
X	X	6511–6714	67	Essential tail gene	98	67	
Y	Y	6718–6999	93	Holin; essential for host cell lysis	99	93	
K	K	6999–7496	165	Endolysin; essential for host cell lysis	100	165	
lysA	LysA	7511–7936	141	Nonessential; affects timing of lysis	92	141	
lysB	LysB	7924–8349	141	Nonessential; affects timing of lysis	95	141	
R	R	8457–8924	155	Essential tail gene; tail completion	98	155	
S	S	8917–9369	150	Essential tail gene; tail completion	98	150	
V	V	9436–10071	211	Baseplate assembly protein	98	211	
W	W	10068–10415	115	Baseplate assembly protein	100	115	
J	J	10420–11328	302	Baseplate assembly protein	99	302	
I	I	11321–11851	176	Baseplate assembly protein	93	176	
H	H	11862–14603	913	Probable tail fiber protein	35	669	Mosaic gene
G	G	14607–15134	175	Probable tail fiber assembly protein	94	175	
orf20	Orf20	c15286–16065	259	Unknown	NA	NA	Not present in P2
FI	FI	16466–17656	396	Essential tail protein; tail sheath	94	396	
FII	FII	17669–18187	172	Essential tail protein; tail tube	95	172	
E+E′	E+E′	18245–18499, 18499–18672	142	Essential tail protein (contains an artificial frameshift like in P2)	97	142	
T	T	18665–21112	815	Essential tail protein; putative tail length	98	815	
U	U	21127–21606	159	Essential tail protein	99	159	
D	D	21606–22769	387	Essential tail protein	97	387	
ogr	Ogr	22850–23068	72	Positive regulator of P2 late gene expression	98	72	
int	Int	c23342–24322	326	Integrase	51	337	Differences throughout
C	C	c24392–24685	97	Immunity repressor	42	99	Differences throughout
cox	Cox	24807–25094	95	Repressor of Pc; required for prophage excision; inhibits integration; activates p11 promoter of satellite phage P4	36	91	Differences throughout
orf78	Orf78	25097–25267	56	Unknown	100	56	
B	B	25264–25764	166	Essential protein; DNA replication; required for lagging strand synthesis	98	166	
orf80	Orf80	25828–26052	74	Unknown	98	74	
orf81	Orf81	26052–26351	99	Unknown	87	100	
orf82	Orf82	26354–26578	74	Unknown	94	74	
orf83	Orf83	26575–26850	91	Unknown	97	91	
A	A	26840–29125	761	Essential protein; DNA replication; makes site-specific nick at origin of replication	97	761	
orf91	Orf91	29122–29574	150	Unknown	49	109	75 aa are 98% identical
orf40	Orf40	c29530–30522	330	Unknown	NA	NA	Not present in P2

[a] c, complementary strand.

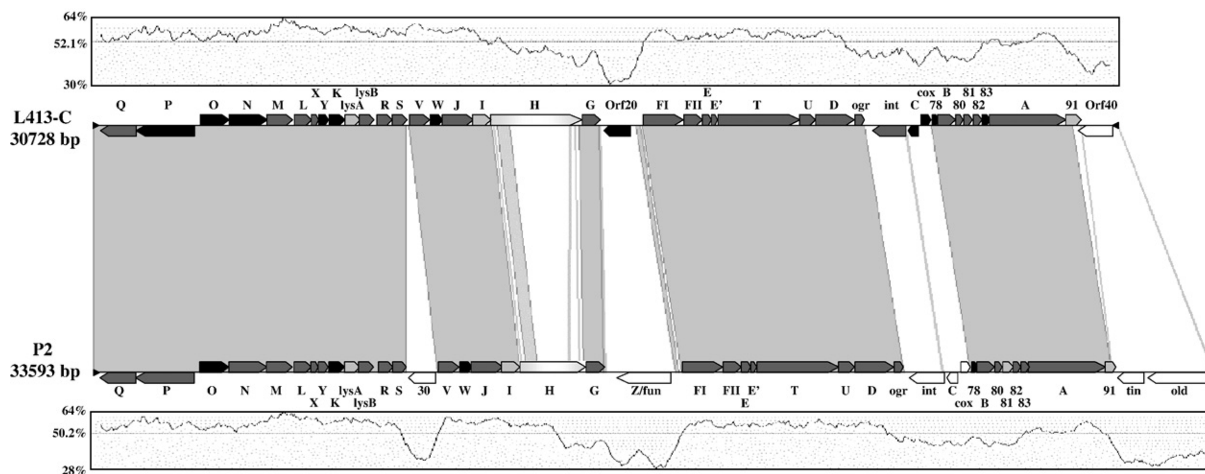

Fig. 1. Structural organization of the L-413C genome and alignment with P2. The L-413C genome shows a high degree of similarity with the P2 genome; the gene organization of both genomes and their regions of similarity is shown, together with G+C content distribution. L-413C protein-coding genes are colored with respect to their similarity to proteins in the nonredundant (NR) sequence database, while those in P2 are colored with respect to their similarity to L-413C homologs (Black, 100% identity hits; dark gray, >94%; light gray, >85%; white, <55%). The H proteins are shaded differently to highlight the mosaic nature of the L-413C homologue. Note: for P2 *orf91*, it was shaded light gray because the similarity only extended through half of the L-413C homologue, even though it was 98%, over 75 amino acids (see text). Shading extending between the two genomes indicates the regions of high similarity. G+C plots (%, 500 base pair window) are shown above and below the genomes displaying regions that are markedly different from the genome average (indicated, line through the plot).

encoded in P2-like prophages found within the genomes of pathogenic *E. coli*: a phage holin responsible for bacterial membrane lysis and found within the enteroinvasive *E. coli* (EIEC) strain 53638 (accession no. ZP_00736273) is identical to the L-413C Y protein, while the Orf83 ortholog, a protein of unknown function (accession no. ZP_00703215) is found within the enterotoxigenic *E. coli* (ETEC) strain E24377A.

Coding potential and similarity with P2

Only five CDSs (Orf20, Int, C, Cox, and Orf40) do not have highly similar orthologs in the original sequenced P2 genome (accession AF063097) (see Fig. 1 and Table 3). Of the 34 CDSs that have clear orthologs in the original P2 sequence, the majority (29) share ≥94% identity throughout the length of the protein, while 5 CDSs (H, I, LysA, Orf81, and Orf91) display greater divergence from P2. Of these more divergent proteins, the most striking difference between L-413C and P2 occurs at the *H* locus that codes for the distal portion of the tail fiber and is discussed in the "H tail fiber protein" section below. Both the tail formation protein I (176 aa), which shares 93% identity with its P2 ortholog, and the lysis control protein LysA (141 aa), which shares 92% identity with P2 LysA, harbor several amino acid substitutions, and of these a greater number are nonconservative substitutions that may be indicative of proteins under selective pressure. An even more divergent protein, Orf81 (99 aa), whose P2 ortholog is only 87% identical, does have a highly similar (98% identical) ortholog (APECO1_4462) in the recently published genome of the avian pathogenic *E. coli* strain APEC O1 (Johnson et al., 2007). Another protein of unknown function, the 150-aa Orf91, appears to be truncated in both P2 (75 aa, 98% identical) and Wφ (92 aa, 98% identical); however, a full-length ortholog (Orf151, 89% overall similarity) is found in the P2-like prophage P2-EC31 (Odegrip et al., 2006).

L-413C differences in nonessential, variable regions

Several major differences, including the 5 CDSs with no P2 orthologs, are apparent in the whole genome alignments with P2 (Fig. 1) and Wφ. Compared with the original P2 genome, L-413C is missing the nonessential, AT-rich *orf30* gene which appears to have been inserted between the genes encoding the tail gpS and baseplate gpV proteins in P2 (Nakayama et al., 1999). Similarly, two other AT-rich P2 variable regions shown to be involved in lysogenic conversion (Calendar et al., 1998) have been replaced with alternative coding sequences in L-413C. The multivariable region corresponding to the P2 *fun/z* locus has been replaced with a putative lysogenic conversion gene *orf20*, whose product is identical to Orf1 found in enterobacteriophage P2-EC64 (Nilsson et al., 2004). Likewise, the variable P2 region that includes the *old* and *tin* genes has been replaced in L-413C with *orf40*, which encodes a 330-aa conserved hypothetical protein that is most highly similar to gp98 (33% identity, 57% similarity) from Wφ (which has also replaced the *old* and *tin* locus) and to a hypothetical protein (55% identity, 72% similarity, accession no. ZP_01511320) in the genome of *Burkholderia phytofirmans* PsJN, whose gene is found in a bacteriophage-related gene cluster.

A fourth P2 variable region consists of an integrase *int*, along with genes involved in immunity, *c* and *cox*. While the encoded products do share sequence similarity (98%, 55%, and 81%) with Wφ Int, C, and Cox proteins, respectively, they are much more divergent from those encoded by the P2 genome (see Table 3 and Fig. 1). In contrast, identical Cox proteins are found in bacteriophage HK109 and prophage P2-EC64 (Nilsson and Haggard-Ljungquist, 2001; Nilsson et al., 2004), while identical C proteins are found to be encoded by many other P2-like phages (Karlsson et al., 2006; Nilsson and Haggard-Ljungquist, 2001; Nilsson et al., 2004).

H tail fiber protein

As mentioned above, one of the more striking and interesting features in the L-413C genome lies in the H locus, which codes for the distal part of the tail fiber. Protein H in P2 and Wφ consists of 669 amino acids while the ortholog in L-413C consists of 913 amino acids and shares only 35% overall identity with P2 and Wφ. Additional BLAST searches reveal a mosaic pattern for the L-413C H protein with four distinct regions (see Fig. 2). Two of these regions are homologous to the P2/Wφ H proteins; the amino-terminal 207 amino acids display ≥91% similarity with the corresponding regions of the H proteins, while the carboxyl-terminal 137 amino acids (aa 777–913) exhibit >59% similarity to the C-terminal 193 amino acids of the P2/Wφ H proteins. The remaining middle portion of the L-413 H protein is not homologous to P2 and appears to be itself a mosaic with greatest similarity to other enterobacteriophage tail fiber proteins. A third, 465 amino acid region consisting of amino acids 226–690, displays homology to only two bacteriophage proteins at 61% similarity: the distal tail fiber subunits of phages RB32 and T6, both belonging to the T4-family bacteriophages. The fourth and last remaining portion of the L-413C H protein is more loosely defined, having homology to a number of putative bacteriophage tail proteins encoded within *E. coli* or *Shigella* bacteriophage-like genomic regions. For example, amino acids 660–863 are 93% similar to the 203 amino acid long putative bacteriophage tail protein (SSON_1887) in the *Shigella sonnei* Ss046 genome (Yang et al., 2005), amino acids 677–771 are 88% similar to tail fiber fragment Mup52 of bacteriophage Mu (Morgan et al., 2002), amino acids 674–913 are 86% similar to a hypothetical protein (accession no. ZP_00709157) in entero-pathogenic *E. coli* (EPEC) strain B171, and amino acids 599–771 are 76% similar to a putative H protein (UTI89_C0924) in the recently released uropathogenic *E. coli* (UPEC) strain UT01 (Chen et al., 2006). The fact that the mosaic, or modular, nature of tail fiber proteins in other dsDNA bacteriophages has been shown to affect host cell receptor binding properties in these phages (Haggard-Ljungquist et al., 1992) led us to investigate the possibility that the mosaic structure of the L-413C H protein may be responsible for its ability to discriminate between *Y. pestis* and its progenitor *Y. pseudotuberculosis*.

EcoKI restriction sites

To ascertain whether restriction sites in the phage sequence may account for the enhanced sensitivity of L-413C to restriction in *E. coli* K-12 derivatives, in relation to P2 (see Table 2), we screened L-413C, P2, and Wφ DNA for the presence of *Eco*KI sites, AAC(N6)GTGC, or GCAC(N6)GTT. While P2 and Wφ carry unique target sites for the *Eco*KI restriction system (with coordinates 27624–27636 and 25816–25828, respectively), L-413C has three times more *Eco*KI sites (located at 13342–13354, 17487–17499, and 26430–26442).

Preadsorption experiments

To determine whether the specificity of L-413C was indeed due to specific binding to *Y. pestis* cells and not due to a simple failure by the part of *Y. pseudotuberculosis* to generate viable phage progeny particles, we conducted a series of preadsorption experiments. When L-413C phage was preadsorbed with *Y. pseudotuberculosis* at a multiplicity of infection (MOI) of 1.0, between 70% and 100% of the phage remained in the supernatant. In contrast, when the phage was incubated (at the same MOI) with *Y. pestis* prior to infection, only 1–2% of the virions remained in the supernatant (see Table 4).

The large reduction in free phage particles when pretreated with *Y. pestis* would indicate that most of the phage are being removed from the suspension by binding to *Y. pestis* cells during pretreatment. The almost negligible reduction in titer when the phage was pretreated with *Y. pseudotuberculosis* indicates that the virions are not binding to the *Y. pseudotuberculosis* cells.

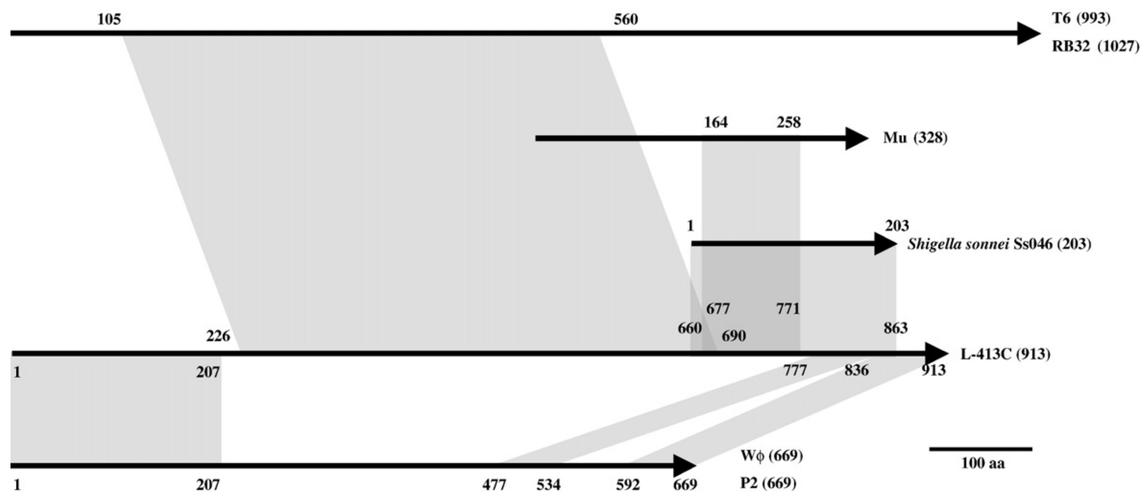

Fig. 2. Alignments of the mosaic L-413C H tail fiber protein with other tail fiber proteins. The best NR database protein hits (arrows) to the sections of the L-413C H protein (1–913 arrow) are shown. While the N- and C-termini display high similarity to phages P2 and Wφ, a large section corresponding to the middle of the H protein more closely resembles the large subunit distal tail fiber of T4-like enterobacteriophages T6 and RB32. The remaining region displays similarity to a number of bacteriophage or bacterially encoded phage tails (proteins in bacteriophage Mu and *S. sonnei* Ss046 are shown). Gray shading represents the regions of similarity and numbers correspond to the locations within each protein (see text for more detail). Source genome is indicated to the right of the proteins, length in amino acids in brackets.

Table 4
Preadsorption experiments with L-413C phage

Phage preincubated with	Remaining phage particles in plaque-formin unit and percent							
	Experiment I		Experiment II		Experiment III		Average percent (±SD)	
	Phage amount		Phage amount		Phage amount			
None (control)	1.7×10^7	2.4×10^8	1.8×10^7	2.1×10^8	4.3×10^7	2.6×10^8		
Y. pseudotuberculosis cells	1.6×10^7, 94.1%	2.5×10^8, 100%*	1.6×10^7, 88.9%	1.7×10^8, 81.0%	6.0×10^7, 100%*	1.8×10^8, 69.2%	94.3% (±5.6%)	83.4% (±15.5%)
Y. pestis cells	1.8×10^5, 1.1%	3.2×10^6, 1.3%	2.3×10^5, 1.3%	2.2×10^6, 1.0%	7.8×10^5, 1.8%	5.2×10^6, 2.0%	1.4% (±0.4%)	1.5% (±0.5%)

* Percentages were rounded to 100% when the number of estimated remaining phage was greater than that remaining in the control.
A fixed amount (ca. 3×10^7 or ca. 3×10^8) of CsCl-banded L-413C phage was preincubated in LB containing 1 mM $CaCl_2$ with a fixed amount (ca. 10^8) of either *Y. pseudotuberculosis* or *Y. pestis* cells for 15 min at 30 °C. The phage-containing supernatant was then collected and titrated against *Y. pestis* cells for the determination of the titer of the phage remaining in the supernatant. Percentages indicate the number of phage that was not adsorbed to the cells.

Therefore, it is likely that the receptors on *Y. pestis* recognized and bound by L-413C either do not exist on *Y. pseudotuberculosis* or are different in their structure, and by inference, that the changes observed in the H protein of L-413 and that of the corresponding P2 protein are responsible for the altered host specificity displayed by this phage.

Immunity tests

We checked the ability of L-413C to make plaques on *E. coli* strains lysogenized with P2-like phages of eight immunity groups. The lysogenic strains used were as follows (see Table 1): C-117 (P2 prophage, immunity group I), C-119 (P2 Hy *dis*, group II), C-1920 (Wφ, group III), TD204 (HK109, group IV), C-1928 (φD160, group V), C-1917 (φD145, group VI), ECOR4 (a defective P2-like prophage, immunity group VII), and E573 (186, out-group). L-413C made plaques on all of these lysogenic strains except ECOR4. Thus L-413C is insensitive to the immunity of phages in groups I–VI, as well as the immunity of phage 186, which is so different as to be classified in an out-group. We cannot say for certain that L-413C is sensitive to the repressor of immunity group VII, since ECOR4 is a wild strain of *E. coli* with a defective prophage that has not been transferred to C-1a for an isogenic test. The inability of L-413C to make plaques on ECOR4 might be due to abortive interaction with any cellular component, not only the repressor of the defective prophage. The sequences of the repressors and operators for L-413C and those of the P2-related phages (Karlsson et al., 2006) suggest that L-413C is not in immunity group VII, but rather in immunity group IV, represented by HK109. The fact that L-413C makes plaques on the HK109-lysogenic strain suggests that L-413C is an immunity-insensitive mutant of a group IV phage.

Discussion

The unique diagnostic value of L-413C and its potential role providing useful insights into the evolution of enterobacteriophages led us to investigate its genomic sequence. L-413C is undoubtedly closely related to the sequenced enterobacteriophages Wφ and P2, thus confirming previous ultrastructural characterization results (presence of an isometric head and contractile tail) (Larina and Konnov, 1980) that had placed L-413C in the family Myoviridae, morphotype A1 (Ackermann, 2003).

As observed in other P2-like bacteriophages, L-413C appears to have evolved by a combination of horizontal transfer events and recombination (Haggard-Ljungquist et al., 1992; Karlsson et al., 2006; Nilsson and Haggard-Ljungquist, 2001; Nilsson et al., 2004). Of 39 encoded proteins in L-413C, 34 display significant similarity with P2 proteins. All five of the remaining genes lie within three nonessential, variable regions of the P2-like genomes. Three (Int, C, and Cox) share some level of similarity (55–98%) to homologs in Wφ (of which two have identical orthologs in other P2-like phages). A fourth protein (Orf20) is not found in either P2 or Wφ but has an identical ortholog of unknown function in the ECOR prophage P2-EC64. This latter replacement comes at the expense of the nonessential P2 *Z(fun)* locus, which is responsible for resistance to phage T5 (Calendar et al., 1998) and sensitivity to 5-fluorouracil (Bertani, 1964). Gene *orf40* whose product best BLAST matches are to hypothetical proteins in Wφ (33% identity) and *B. phytofirmans* (55% identity), replaces the *tin* and *old* variable gene region of P2, which encodes resistance to phages T2 (Mosig et al., 1997) and λ (Lindahl et al., 1970), respectively. Thus, three of P2's original nonessential genes confer resistance to other phages and are replaced by genes of unknown function in L-413C. A different P2-related phage, enterobacteriophage 186, has also replaced these phage-resistance genes, but with genes whose functions are required for ultraviolet induction of their prophage form (Shearwin et al., 1998). Moreover, Odegrip et al. (2006) have analyzed the *tin*/*old* nonessential region in six other P2-related phages and found that they were all variable, each encoding a different set of genes (Odegrip et al., 2006). One of these gene products was found to encode a functional reverse transcriptase and blocks infection by phage T5. Given that phage 186 as well as P2 and other P2-like genomes have acquired useful genes in these nominally nonessential variable regions, the two unique genes of unknown function in L-413C, *orf20* and *orf40*, may also encode functions that provide a selective advantage for either the phage or the lysogenic host.

Homologous recombination has been shown to also be involved in the evolution of immunity and host chromosome integration site determinants, Int, C, and Cox (Karlsson et al., 2006). While the Int protein of L-413C is 98% similar to that of

Wφ, both C and Cox are less similar (at 55% and 81%, respectively) to Wφ, yet have 100% identical orthologs in other sequenced P2-like phages (Karlsson et al., 2006). Using *E. coli* strains lysogenized with P2, Wφ and six other phages belonging to eight different immunity groups, we showed here that L-413C is likely to be an immunity-insensitive mutant. Unfortunately, this hypothesis cannot be checked, since we do not have the parent, turbid-plaque-forming phage for comparison.

Two additional L-413C proteins (H and Orf91) appear to be chimeric versions of proteins found in P2 and other P2-like phages. The protein of unknown function Orf91 is larger than its P2 and Wφ homologs and is more closely related to the ECOR prophage P2-EC31. It lies directly 5′ to the *tin*/*old* locus and variation in this L-413C gene is likely related to the variation seen in *tin* and *old*. The finding that the L-413C tail fiber protein H is made up of four regions, with N- and C-termini most similar to P2-like phages (Fig. 2), a large middle region most highly similar to T4-like phages T6 and RB32, and a smaller middle section most similar to tail fiber proteins in phage Mu and prophages of *S. sonnei* and *E. coli*, is consistent with the mosaic derivation of this protein, as previously observed in other P2-like phages (Haggard-Ljungquist et al., 1992). Furthermore, since the host range of the P2-like tailed phages is determined by the tail fibers, the H tail fiber protein of phage L-413C is likely to control the host specificity as well. We postulate that the specific substitutions observed in protein H are responsible for host range mutation that led to the particular specificity of L-413C for *Y. pestis* (compared with *Y. pseudotuberculosis*). Thus, L-413C is a member of the P2 group that has undergone the loss and replacement of some nonessential but useful genes and that has undergone recombinational events at the level of the tail fiber protein H that have led to the new host-range specificity displayed by this unique yersiniaphage.

Consistent with this idea, preadsorption experiments with L-413C binding to either *Y. pestis* or *Y. pseudotuberculosis* cells (Table 4) demonstrated that the specificity shown by this phage towards *Y. pestis* is due to its unequivocal binding to *Y. pestis* cells and not to other factors such as failure to inject, replicate or escape from *Y. pseudotuberculosis* cells. The mosaic nature of bacteriophage tail fiber proteins has been elegantly demonstrated in the case of P1 (Sandmeier et al., 1992) and P2, in which the fiber protein was shown to be a mosaic combining parts of the tail fibers of lambda, Mu, P1, P2, T2, and T4 (Haggard-Ljungquist et al., 1992). In the case of L-413C, one can speculate that the unique specificity of L-413C could have emerged, not from a long co-evolution with *Y. pestis*, but rather as an unintended result of the ongoing co-evolution of P2-like and T6-like enterophages in *E. coli*, *Shigella*, and other enterobacteria. In such a scenario, *Y. pestis* would have served as a well-suited vehicle to pick, to propagate, and to maintain this hybrid phage.

In this work, we have confirmed the high diagnostic value of L-413C. This phage efficiently lysed *Y. pestis* and restriction-deficient strains of *E. coli*, but not *Y. pseudotuberculosis* and *E. coli* $r_k^+m_k^+$ strains. Our data are in agreement with the results of previous work that tested 103 *E. coli* strains, the vast majority of which were fresh clinical isolates (Larina, 1976). Of them, only C-85, a streptomycin-resistant derivative of a "restrictionless" strain, *E. coli* C (Bertani and Bertani, 1970), was lysed by L-413C. Our experiments with isogenic *E. coli* C600 r_k^+ and r_k^- variants showed that L-413C is highly sensitive to *Eco*KI restriction, when compared to P2. One of the reasons for such an enhanced sensitivity is quite likely the presence of a greater number of *Eco*KI site in L-413C (three times as many) as those found in P2 and Wφ.

As for the question of the possible receptors in *Y. pestis* targeted by L-413C, we can only conjecture. It is known that the receptor for phage P2 of *E. coli* and *Shigella* is located in the first glucose of outer core of the bacterial lipopolysaccharide (LPS) (Hannecart-Pokorni et al., 1976). The receptor for phage T6 (the other bacteriophage that possesses a tail fiber H gene with an extensive region of homology with L-413C) is the outer membrane protein Tsx, a nucleoside-specific channel (Manning and Reeves, 1976). However, analyses among those *Y. pestis* and *Y. pseudotuberculosis* genomes sequenced thus far have not revealed the presence of the *tsx* (*nupA*) gene (Gu et al., 2006). Additionally, we were unable to find in any *Yersinia* genome any sequence similarity to the small domain near the *E. coli* Tsx C-terminus which serves as the T6 receptor area (Schneider et al., 1993). Thus, Tsx-related sequences are unlikely to operate as receptor regions for the L-413C phage and may play instead a structural role.

Our tests have shown that L-413C lyses *Y. pestis* at both 28 and 37 °C, thus excluding the capsular antigen as the phage receptor candidate since it is only produced at 37 °C. Given that the lack of O-antigen in the LPS structure is one of the major differences between *Y. pestis* and *Y. pseudotuberculosis* (with the latter having a smooth LPS and being resistant to L-413C), it is interesting to speculate whether the *Y. pestis* LPS lacking O-specific polysaccharide side-chains (rough type; for review, see Knirel et al., 2006) could constitute the L-413C receptor. Using isogenic O-antigen positive and negative strains of *Y. pseudotuberculosis*, we observed a clear correlation of P2 plating efficiency with the presence of rough-form LPS at growth temperatures of 25 and 37 °C. Our data suggest that the O-antigen shields a P2 phage receptor in *Y. pseudotuberculosis*, which is likely to lie in a stable, temperature-independent part of core LPS. Since P2-induced lysis of *Y. pestis* was temperature-dependent and less efficient in comparison with a rough strain of *Y. pseudotuberculosis*, the P2 phage receptor in *Y. pestis* may differ from that of *Y. pseudotuberculosis*. As for L-413C, one can only say now that its receptor in *Y. pestis* is represented by a temperature-independent surface structure, which is probably absent from *Y. pseudotuberculosis*. Unraveling of the exact mechanism of interaction of L-413C and its host, *Y. pestis*, and the identification of the L-413C receptor in this organism would provide a solid basis for the development of fast and specific detection systems for the plague bacillus.

Materials and methods

Bacteria

The bacterial strains used in this work are listed in Table 1.

Phage growth and DNA isolation

As an initial stock, we used an ampoule of an L-413C preparation obtained from the M. Aikimbayev's Kazakh Scientific Center for Quarantine and Zoonotic Diseases, Almaty, Kazakhstan. The phage propagation and titration was performed on *Y. pestis* PKR133 or KM218 (Table 1). A highly active lytic clone was selected from a large clear single plaque on strain PKR133 and passaged several times in the same strain. Large-scale preparation of the phage particles and DNA extractions were performed using a slight modification to the methods described for bacteriophage λ (Sambrook et al., 1989), using a low multiplicity of infection (0.1–0.4), and the Yamamoto purification scheme. Phage particles were concentrated from 1 M NaCl 10% PEG 8000 solution by centrifugation in a Beckman SW27 rotor at 11,000×*g* for 10 min at 4 °C and resuspended in SM buffer. The bacteriophage suspension was treated with chloroform, DNase, and RNase followed by lysis with SDS–proteinase K solution. The DNA was purified with equilibrated phenol, phenol–chloroform, and chloroform and then precipitated with ethanol.

Library construction and sequencing

Phage DNA was subjected to mechanical physical breaking using a hydroshear (Gene Machines; Genomics Instrumentation Services, Inc., San Carlos, CA) to yield 1- to 2-kb-sized fragments. These were end-repaired and cloned into dephosphorylated, *Sma*I-digested pUC18 (Amersham Pharmacia Biotech., Piscataway, NJ) and M13mp18 vectors (Novagen, EMD Biosciences, Inc., La Jolla, CA). A 10-fold redundant pUC-based library and a 5-fold M13-based library were constructed using DH5α and DH5α F′IQ (Invitrogen Corporation, Carlsbad, CA) cells. Three 96-well plates of M13 clones (288 clones) were sequenced using Big Dye terminator chemistry, ABI PRISM 373 or 377 sequencers (PE Applied Biosystems, Foster City, CA), and −21m13 forward primer (Operon Technologies, Inc., Huntsville, AL). Three additional 96-well plates of pUC-based clones were sequenced in the same fashion using both the forward and the reverse, m13rp1 primers. Complete assembly was obtained by joining two initial contigs by means of a PCR product obtained from the original phage template. Additional sequencing was conducted on PCR products to obtain a high quality sequence.

Annotation and analysis of sequences

The final sequence was searched against the current protein and nucleotide databases (http://www.ncbi.nlm.nih.gov/) by using the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990). Bacteriophage P2, along with Wφ, were used as reference for genome alignments and comparisons due to their high level of similarity with L-413C. Sequence alignments and motif searches were conducted using the GeneWorks, 1995 Release (Intelligenetics, Mountain View, CA), the web-based program MOTIF (www.motif.genome.ad.jp), BLAST (Altschul et al., 1990), the ClustalW multiple alignment program (Thompson et al., 1994), the Artemis Comparison Tool (The Sanger Centre, Cambridge, UK), the Windows 32 EditSeq 6.1 (DNASTAR Inc., Madison, WI), and the Alion (Nevill-Manning et al., 1997) (http://motif.stanford.edu/alion/).

Phage adsorption experiments

L-413C phage for the preadsorption experiments was propagated on *Y. pestis* D88 strain (Java, Indonesia, biovar Orientalis). The phage-infected cells were grown in LB supplemented with 0.1% glucose, 1.6 mM $MgCl_2$, and 0.5 mM $CaCl_2$, precipitated with NaCl and PEG8000, treated with DNAse I in 10 mM $MgCl_2$, 10 mM Tris–HCl (pH 7.5), and 1% ammonium acetate with subsequent purification of the phage particles by centrifugation in a CsCl density gradient. Fixed amounts (3×10^7 or 3×10^8 pfu) of CsCl-banded L-413C phage were preincubated with a fixed amount (10^8) of *Y. pseudotuberculosis* cells for 15 min at 26 °C. Following incubation, the supernatant from the spun cells was titered by incubating it with 10^8 *Y. pestis* cells for 15 min followed by plating onto BHI (Becton-Dickinson, Franklin Lakes, NJ) plates. As a control, the same amount of phage was incubated with 10^8 *Y. pestis* cells for 15 min at 26 °C prior to titering. A comparison between the titers of phage preadsorbed with *Y. pestis* and phage preadsorbed with *Y. pseudotuberculosis* against the titer of the phage with no pretreatment indicated whether the phage bound receptors on *Y. pseudotuberculosis*.

Phage lysis tests

For the spot tests, *Y. pestis* strains were plated on BHI agar at 28 °C for 48 h while *E. coli* cultures were plated on LB agar (Becton-Dickinson) at 37 °C for 24 h. The cultures were then restreaked on the corresponding agar plates to obtain solid lawns, and 25 μl aliquots of L-413C or P2 phage suspensions (both at concentration of ca. 10^7 pfu/ml) was applied in the center of seeded agar sectors and the plates were incubated at both 28 °C and 37 °C for 18 h. For the efficiency of plating assays, the bacterial strains were grown in LB (*E. coli*) or BHI (*Y. pestis* and *Y. pseudotuberculosis*) at 37 °C and/or 25 °C overnight without shaking. Culture (0.25 ml) was added to varying amounts of phage, and $CaCl_2$ was added to 5 mM. The mixture was incubated at the same temperature for 10 min, then plated in 2.5 ml LB or BHI soft (0.7%) agar on 1.5% LB or BHI agar plates and incubated overnight at the corresponding temperature.

Phage immunity assays

About 100 pfu of L-413C was mixed with 0.2 ml of each overnight lysogenic culture (Table 1) grown in LB at 37 °C without aeration, then preincubated for 10 min at 37 °C in the presense of 5 mM $CaCl_2$ and plated on L agar at 37 °C.

Nucleotide sequence accession number

Nucleotide sequence data for L-413C have been deposited in GenBank under accession no. NC_004745.

Acknowledgments

We thank Ms. L.A. Novichkova for excellent technical assistance, Drs. M.P. Nikolich, D.A. Portnoy, M. Skurnik, B.J. Hinnebusch, G. Bertani, E. Haggård-Ljungquist, J.B. Egan, and P. Berg for kindly providing bacterial strains, and Drs. G. Bertani and E. Haggård-Ljungquist for their helpful discussions. This work was implemented under the auspices of the U.S. Department of Energy under contract no. W-7405-Eng-48.

References

Ackermann, H.W., 2003. Bacteriophage observations and evolution. Res. Microbiol. 154, 245–251.

Advier, M., 1933. Etude d'un bactériophage antipesteux. Bull. Soc. Pathol. Exot. 26, 94–99.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410.

Andrews, G.P., Heath, D.G., Anderson Jr., G.W., Welkos, S.L., Friedlander, A.M., 1996. Fraction 1 capsular antigen (F1) purification from *Yersinia pestis* CO92 and from an *Escherichia coli* recombinant strain and efficacy against lethal plague challenge. Infect. Immun. 64, 2180–2187.

Anisimov, A.P., Lindler, L.E., Pier, G.B., 2004. Intraspecific diversity of *Yersinia pestis*. Clin. Microbiol. Rev. 17, 434–464.

Aparin, G.P., Golubinskii, E.P., 1989. Plague Microbiology: Manual. Irkutsk State University, Irkutsk.

Appleyard, R.K., 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K-12. Genetics 39, 440–452.

Arutyunov, Y.I., 1970. Biological characteristics of plague and pseudotuberculosis bacteriophages. Zh. Mikrobiol. Epidemiol. Immunobiol. 8, 106–111.

Arutyunov, Y.I., Novoseltsev, N.N., 1970. About the problem of nonspecificity of plague phages. Probl. Particularly Dangerous Infect., vol. 19, pp. 107–111.

Bachmann, B.J., 1996. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12. In: Neidhardt, F.C. (Ed.), *Escherichia coli* and *Salmonella*. ASM Press, Washington D.C, pp. 2460–2488.

Bertani, L.E., 1957. The effect of the inhibition of protein synthesis on the establishment of lysogeny. Virology 4, 53–71.

Bertani, L.E., 1964. Lysogenic conversion by bacteriophage P2 resulting in an increased sensitivity of *Escherichia coli* to 5-fluorodeoxyuridine. Biochim. Biophys. Acta 87, 631–640.

Bertani, L.E., Bertani, G., 1970. Preparation and characterization of temperate, non-inducible bacteriophage P2 (host: *Escherichia coli*). J. Gen. Virol. 6, 201–212.

Bertani, L.E., Bertani, G., 1971. Genetics of P2 and related phages. Adv. Genet. 16, 199–237.

Bobrov, A.G., Kirillina, O.A., Filippov, A.A., Kutyrev, V.V., 1999. Restriction mapping of DNA of plague diagnostic phages of Pokrovskaya and L-413C. Probl. Particularly Dangerous Infect., vol. 79, pp. 138–144.

Brubaker, R.R., 1969. Mutation rate to nonpigmentation in *Pasteurella pestis*. J. Bacteriol. 98, 1404–1406.

Burrows, T.W., Bacon, G.W., 1960. V and W antigens in strains of *Pasteurella pseudotuberculosis*. Br. J. Exp. Pathol. 41, 38–44.

Calendar, R., Yu, S., Myung, H., Barreiro, V., Odegrip, R., Carlson, K., Davenport, L., Mosig, G., Christie, G.E., Haggård-Ljungquist, E., 1998. The lysogenic conversion genes of coliphage P2 have unusually high AT content. In: Syvanen, J.M., Kado, C. (Eds.), Horizontal Gene Transfer, Mechanisms and Implications. Chapman-Hall, London, pp. 241–252.

Cavanaugh, D.C., Quan, S.F., 1953. Rapid identification of *Pasteurella pestis*: using specific bacteriophage lyophilized on strips of filter paper, a preliminary report. Am. J. Clin. Pathol. 23, 619–620.

Chen, S.L., Hung, C.S., Xu, J., Reigstad, C.S., Magrini, V., Sabo, A., Blasiar, D., Bieri, T., Meyer, R.R., Ozersky, P., Armstrong, J.R., Fulton, R.S., Latreille, J.P., Spieth, J., Hooton, T.M., Mardis, E.R., Hultgren, S.J., Gordon, J.I., 2006. Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: a comparative genomics approach. Proc. Natl. Acad. Sci. U. S. A. 103, 5977–5982.

Chu, M.C., 2000. Laboratory Manual of Plague Diagnostic Tests. Centers for Disease Control and Prevention, Atlanta.

Dennis, D.T., Gage, K.L., Gratz, N., Poland, J.D., Tikhomirov, E., 1999. Plague Manual: Epidemiology, Distribution, Surveillance and Control. World Health Organization, Geneva.

d'Herelle, F., 1926. The Bacteriophage and Its Behavior. Williams & Wilkins Co., Baltimore.

Dhillon, E.K., Dhillon, T.S., Lam, Y.Y., Tsang, A.H., 1980. Temperate coliphages: classification and correlation with habitats. Appl. Environ. Microbiol. 39, 1046–1053.

Domaradskij, I.V., 1998. Plague. Meditsina Press, Moscow.

Gage, K.L., Kosoy, M.Y., 2005. Natural history of plague: perspectives from more than a century of research. Annu. Rev. Entomol. 50, 505–528.

Garcia, E., Elliott, J.M., Ramanculov, E., Chain, P.S.G., Chu, M.C., Molineux, I.J., 2003. The genome sequence of *Yersinia pestis* bacteriophage φA1122 reveals an intimate history with the coliphage T3 and T7 genomes. J. Bacteriol. 185, 5248–5262.

Glover, S.W., Kerszman, G., 1967. The properties of a temperate bacteriophage Wφ isolated from *Escherichia coli* strain W. Genet. Res. Camb. 9, 135–139.

Gu, J., Neary, J.L., Sanchez, M., Yu, J., Lilburn, T.G., Wang, Y., 2006. Genome evolution and functional divergence in *Yersinia*. J. Exp. Zoolog. B. Mol. Dev. Evol. 308, 37–49.

Gunnison, J.B., Larson, A., Lazarus, A.S., 1951. Rapid differentiation between *Pasteurella pestis* and *Pasteurella pseudotuberculosis* by action of bacteriophage. J. Infect. Dis. 88, 254–255.

Haggard-Ljungquist, E., Halling, C., Calendar, R., 1992. DNA sequences of the tail fiber genes of bacteriophage P2: evidence for horizontal transfer of tail fiber genes among unrelated bacteriophages. J. Mol. Biol. 174, 1462–1477.

Hanahan, D., 1985. Techniques for transformation of *E. coli*. In: Glover, D.M. (Ed.), DNA Cloning, vol. 1. IRL Press, Oxford, pp. 109–135.

Hannecart-Pokorni, E., Godard, C., Beumer, J., 1976. Chemotypes of *Shigella flexneri* R mutants and related phage receptors. II. Localization of phage receptors (author's translation). Ann. Microbiol. (Paris) 127, 15–24.

Hertman, I., 1964. Bacteriophage common to *Pasteurella pestis* and *Escherichia coli*. J. Bacteriol. 88, 1002–1005.

Imamaliev, O.G., Serebryakova, V.G., Anisimova, T.I., Plotnikov, O.P., Sergeeva, G.M., Goldfarb, L.M., Larina, V.S., 1986. Comparative estimation of activity and specificity of diagnostic plague bacteriophages, L-413C, and the Pokrovskaya phage. In: Bektemirov, T.A., Zhouravleva, Y.Z., Litvinova, M.Y. (Eds.), Standards, Strains, and Methods of Control of Bacterial and Viral Preparations. Mechnikov Institute Press, Moscow, pp. 102–106.

Johnson, T.J., Kariyawasam, S., Wannemuehler, Y., Mangiamele, P., Johnson, S.J., Doetkott, C., Skyberg, J.A., Lynne, A.M., Johnson, J.R., Nolan, L.K., 2007. The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. J. Bacteriol. 189, 3228–3236.

Karlsson, J.L., Cardoso-Palacios, C., Nilsson, A.S., Haggard-Ljungquist, E., 2006. Evolution of immunity and host chromosome integration site of P2-like coliphages. J. Bacteriol. 188, 3923–3935.

Kiljunen, S., Hakala, K., Pinta, E., Huttunen, S., Pluta, P., Gador, A., Lönnberg, H., Skurnik, M., 2005. Yersiniophage φR1-37 is a tailed bacteriophage having a 270 kb DNA genome with thymidine replaced by deoxyuridine. Microbiology 151, 4093–4102.

Knapp, W., 1962. Studies with *Pasteurella pseudotuberculosis* and *Pasteurella pestis* phages. Z. Hyg. Infektionskr. 148, 375–382.

Knapp, W., Zwillenberg, L.O., 1964. Morphological differences between *Pasteurella* bacteriophages. Arch. Gesamte Virusforsch. 14, 563–566.

Knirel, Y.A., Dentovskaya, S.V., Senchenkova, S.N., Shaikhutdinova, R.Z., Kocharova, N.A., Anisimov, A.P., 2006. Structural features and structural variability of the lipopolysaccharide of *Yersinia pestis*, the cause of plague. J. Endotoxin Res. 12, 3–9.

Kudryakova, T.A., Makedonova, L.D., Mishan'kin, B.N., Kachkina, G.V., Kazakov, A.M., Sayamov, S.R., 1999. Phage typing of freshly isolated strains of *Yersinia pestis* from Central Caucasian focus. Materials of the Conference "Diagnostics, Therapy, and Prophylaxis of Infectious Diseases. Biotechnology. Veterinary." Ekaterinburg, pp. 114–115.

Kutyrev, V.V., Satybaldiev, N.A., Verenkov, M.S., Bakanurskaya, T.L., Filippov, A.A., Pak, G.Y., Protsenko, O.A., 1987. Changes in properties of *Yersinia pestis* phage resistant cultures and additional tests for their studying. Epidemiology, Molecular Biology and Immunology of *Yersinia pestis*, pp. 90–94. Saratov, pt. 1.

Kutyrev, V.V., Filippov, A.A., Shavina, N.Y., Protsenko, O.A., 1989. Genetic analysis and modeling of the virulence of *Yersinia pestis*. Mol. Genet. Mikrobiol. Virusol. 8, 42–47.

Larina, V.S., 1976. Lysogenic clones of plague bacteria of wild type and characteristics of phages produced by them. Zh. Mikrobiol. Epidemiol. Immunobiol. 1, 119–122.

Larina, V.S., Kishenevskii, A.M., 1973. About isolation of temperate plague phage from the vaccine strain EV NIIEG. Probl. Particularly Dangerous Infect., vol. 32, pp. 87–88.

Larina, V.S., Konnov, N.P., 1980. Electron microscopic study of phages isolated from wild lysogenic clones of *Yersinia pestis*. In: Kondrashin, Y.I., Adamov, A.K., Golubinskii, E.P. (Eds.), Genetics and Biochemistry of Particularly Dangerous Infections. Institute “Microbe” Press, Saratov, pp. 21–24.

Larina, V.S., Anisimov, P.I., Adamov, A.K., 1970. A novel strain of plague bacteriophage for identification of *Pasteurella pestis*. Probl. Particularly Dangerous Infect., vol. 11, pp. 132–136.

Larina, V.S., Rozanova, G.N., Timofeeva, L.A., Logachyov, A.I., Aliev, M.N., Chumachenko, V.D., Mel’nikov, I.F., Zaitsev, V.A., Gramotina, L.I., Jafarov, N.I., 1981. Comparative characterization of specificity of diagnostic plague bacteriophages, L-413C (dry and liquid), and the Pokrovskaya phage. In: Gor’kova, A.V., Adamov, A.K., Avrorov, V.P. (Eds.), Pathological Physiology of Particularly Dangerous Infections. Institute “Microbe” Press, Saratov, pp. 87–90.

Larina, V.S., Sagimbekov, U.A., Dernovaya, V.F., Yusupova, N.A., Sapozhnikov, V.I., Novikov, G.S., Novikova, T.A., Kourganov, V.A., Dmitrovskii, A.M., Vorob’yova, T.V., Sokolov, P.N., 1994. The causes of erroneous estimation of efficiency of diagnostic phage L-413C. Probl. Particularly Dangerous Infect., vol. 75, pp. 85–93.

Lebedeva, S.A., 2000. *Yersinia pestis* variants resistant to diagnostic bacteriophage and problems connected with them. Zh. Mikrobiol. Epidemiol. Immunobiol. 3, 99–104.

Leshkovich, N.L., Arutyunov, Y.I., Tokarev, Y.I., Kirdeev, V.K., 1975. Morphology of plague temperate phages 1701, 1710, and their mutants. Zh. Mikrobiol. Epidemiol. Immunobiol. 10, 31–34.

Lindahl, G., Sironi, G., Bialy, H., Calendar, R., 1970. Bacteriophage λ: abortive infection of bacteria lysogenic for phage P2. Proc. Natl. Acad. Sci. U. S. A. 66, 587–594.

Mandel, M., Berg, A., 1968. Cohesive sites and helper phage function of P2, lambda, and 186 DNA’s. Proc. Natl. Acad. Sci. U. S. A. 60, 265–268.

Manning, P.A., Reeves, P., 1976. Outer membrane of *Escherichia coli* K-12: TSX mutants (resistant to bacteriophage T6 and colicin K) lack an outer membrane protein. Biochem. Biophys. Res. Commun. 71, 466–471.

Molnar, D.M., Lawton, W.D., 1969. Pasteurella bacteriophages sex-specific in *Escherichia coli*. J. Virol. 4, 896–900.

Morgan, G.J., Hatfull, G.F., Casjens, S., Hendrix, R.W., 2002. Bacteriophage Mu genome sequence: analysis and comparison with Mu-like prophages in *Haemophilus*, *Neisseria* and *Deinococcus*. J. Mol. Biol. 317, 337–359.

Mosig, G., Yu, S., Myung, H., Haggård-Ljungquist, E., Davenport, L., Carlson, K., Calendar, R., 1997. A novel mechanism of virus-virus interactions: bacteriophage P2 Tin protein inhibits phage T4 DNA synthesis by poisoning the T4 single-stranded DNA binding protein, gp32. Virology 230, 72–81.

Nakayama, K., Kanaya, S., Ohnishi, M., Terawaki, Y., Hayashi, T., 1999. The complete nucleotide sequence of φCTX, a cytotoxin-converting phage of *Pseudomonas aeruginosa*: implications for phage evolution and horizontal gene transfer via bacteriophages. Mol. Microbiol. 31, 399–419.

Nevill-Manning, C.G., Sethi, K.S., Wu, T.D., Brutlag, D.L., 1997. Enumerating and ranking discrete motifs. Proc. Int. Conf. Intell. Syst. Mol. Biol. 5, 202–209.

Nilsson, A.S., Haggard-Ljungquist, E., 2001. Detection of homologous recombination among bacteriophage P2 relatives. Mol. Phylogenet. Evol. 21, 259–269.

Nilsson, A.S., Karlsson, J.L., Haggard-Ljungquist, E., 2004. Site-specific recombination links the evolution of P2-like coliphages and pathogenic enterobacteria. Mol. Biol. Evol. 21, 1–13.

Novoseltsev, N.N., 1967. About isolation of temperate phages of *Pasteurella pestis*. In: Domaradskij, I.V., Suchkov, Y.G., Gamleshko, H.P., Kanchookh, A.A., Usenko, E.G. (Eds.), Genetics, Biochemistry and Immunochemistry of Particularly Dangerous Infections. Rostov University Press, Rostov-on-Don, pp. 91–98. pt. 1.

Novoseltsev, N.N., Marchenkov, V.I., 1990. *Yersinia pestis* phage of a new serovar. Zh. Mikrobiol. Epidemiol. Immunobiol. 11, 9–12.

Novoseltsev, N.N., Arutyunov, Y.I., Tokarev, S.A., Kirdeev, V.K., 1971. Electron microscopic study of *Pasteurella pestis* phages. Zh. Mikrobiol. Epidemiol. Immunobiol. 3, 16–19.

Novoseltsev, N.N., Marchenkov, V.I., Arutyunov, Y.I., 1994. *Yersinia pestis* phages of serovar 4. Zh. Mikrobiol. Epidemiol. Immunobiol. 6, 9–10.

Ochman, H., Selander, R.K., 1984. Standard reference strains of *Escherichia coli* from natural populations. J. Bacteriol. 157, 690–693.

Odegrip, R., Nilsson, A.S., Haggård-Ljungquist, E., 2006. Identification of a gene encoding a functional reverse transcriptase within a highly variable locus in the P2-like coliphages. J. Bacteriol. 188, 1643–1647.

Pak, G.Y., Satybaldiev, N.A., Bakanurskaya, T.L., Aniskina, G.A., Soorbekov, O.S., 1985. Study of properties of *Yersinia pestis* strains resistant to diagnostic phage L-413C. Zh. Mikrobiol. Epidemiol. Immunobiol. 8, 33–36.

Plotnikov, O.P., Larina, V.S., Kondrashin, Y.I., Vorob’yova, T.V., 1982. Spectrum of lytic activity of bacteriophages isolated from soil of rodents’ holes. In: Anisimov, P.I., Karaseva, Z.N., Konnov, N.P. (Eds.), Selection and Genetics of Causative Agents of Particularly Dangerous Infections. Institute “Microbe” Press, Saratov, pp. 56–61.

Plotnikov, O.P., Konnov, N.P., Goldfarb, L.M., 1985. Structure of some phages capable of propagating in *Yersinia pestis* cells. In: Anisimov, P.I., Konnov, N.P., Naumov, A.V. (Eds.), Problems of Genetics, Molecular Biology and Microbiology of Plague and Cholera. Institute “Microbe” Press, Saratov, pp. 53–58.

Pokrovskaya, M.P., 1929. A plague bacteriophage in dead susliks. Gigiena Epidemiol. 12, 31–34.

Popov, Y.A., Kirillina, O.A., 1982. Structural similarity of plague bacteriophages N 46 and the diagnostic Pokrovskaya one. In: Anisimov, P.I., Vinogradova, N.A., Kutyrev, V.V., Popov, Y.A., Protsenko, O.A. (Eds.), Molecular Biology and Genetics of Causative Agents of Particularly Dangerous Infections. Saratov University Press, Saratov, pp. 58–62. pt.1.

Portnoy, D., Falkow, S., 1981. Virulence-associated plasmids from *Yersinia enterocolitica* and *Yersinia pestis*. J. Bacteriol. 148, 877–883.

Protsenko, O.A., Filippov, A.A., Kutyrev, V.V., 1992. Plasmid heterogeneity in populations of *Yersinia pestis* strains. Mol. Genet. Mikrobiol. Virusol. 3–4, 20–24.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sasaki, I., Bertani, G., 1965. Growth abnormalities in Hfr derivatives of *Escherichia coli* strain C. J. Gen. Microbiol. 40, 365–376.

Sandmeier, H., Iida, S., Arber, W., 1992. DNA inversion regions Min of plasmid p15B and Cin of bacteriophage P1: evolution of bacteriophage tail fiber genes. J. Bacteriol. 174, 3936–3944.

Schneider, H., Fsihi, H., Kottwitz, B., Mygind, B., Bremer, E., 1993. Identification of a segment of the *Escherichia coli* Tsx protein that functions as a bacteriophage receptor area. J. Bacteriol. 175, 2809–2817.

Shashaev, M.A., 1964. Biological characteristics of plague bacteriophages. Zh. Mikrobiol. Epidemiol. Immunobiol. 12, 32–35.

Shearwin, K.E., Brumby, A.M., Egan, J.B., 1998. The Tum protein of coliphage 186 is an antirepressor. J. Biol. Chem. 273, 5708–5715.

Smith, D.A., Burrows, T.W., 1962. Phage and bacteriocin investigations with *Pasteurella pestis* and other bacteria. Nature 193, 397–398.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence

weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Wood, W.B., 1966. Host-specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. 16, 118–133.

Yang, F., Yang, J., Zhang, X., Chen, L., Jiang, Y., Yan, Y., Tang, X., Wang, J., Xiong, Z., Dong, J., Xue, Y., Zhu, Y., Xu, X., Sun, L., Chen, S., Nie, H., Peng, J., Xu, J., Wang, Y., Yuan, Z., Wen, Y., Yao, Z., Shen, Y., Qiang, B., Hou, Y., Yu, J., Jin, Q., 2005. Genome dynamics and diversity of *Shigella* species, the etiologic agents of bacillary dysentery. Nucleic Acids Res. 33, 6445–6458.