Supplementary material for: **Specific detection of *Yersinia pestis* based on receptor binding proteins of phages**

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Supplementary Table S1: Strains, phages and plasmids used in this work.

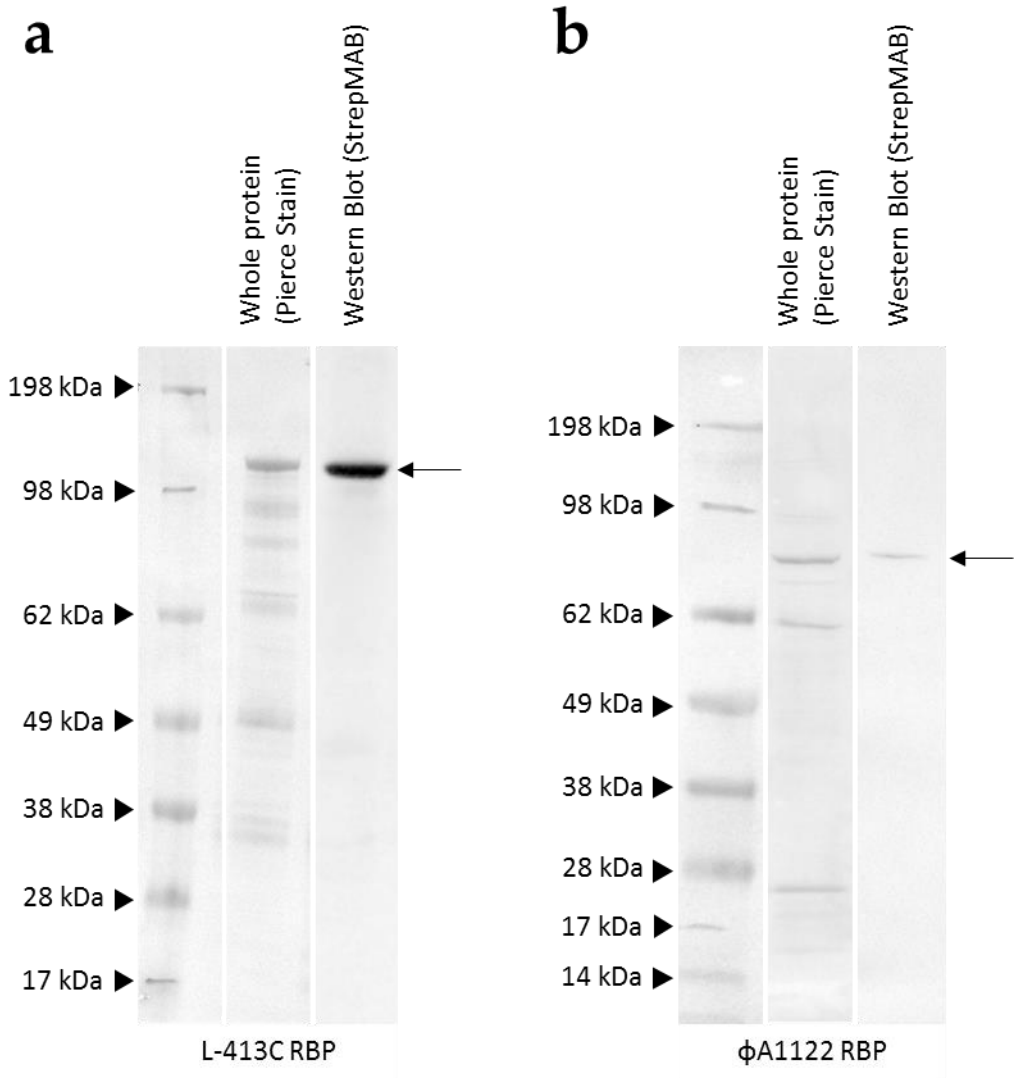
Strain/phage/plasmid	Characteristics	Reference
E. coli BL21 (DE3)	*F⁻ ompT hsdSB ($r_B^-m_B^-$) gal dcm* (DE3)	ThermoFisher Scientific, Darmstadt, Germany
E. coli ArcticExpress™ (DE3)	*E. coli B F⁻ ompT hsdS(r_B^- m_B^-) dcm⁺ Tet^r gal λ* (DE3) *endA Hte [cpn10cpn60 Gent^r]*	Agilent Technologies, Waldbronn, Germany
E. coli NEB Turbo	*F´ proA⁺B⁺ lacI^q ΔlacZ M15/ fhuA2 Δ(lac-proAB) glnV gal R(zgb-210::Tn10)Tet^S endA1 thi-1 Δ(hsdS-mcrB)*	New England Biolabs GmbH, Frankfurt am Main, Germany
Yersinia pestis EV 76	Attenuated deletion mutant (*pgm/hms⁻*), vaccine strain	[1]
Yersinia pestis Kuma	Bv. Antiqua	[2]
Yersinia pestis M23	Bv. Orientalis	[3]
Yersinia pestis TS	Bv. Orientalis	[4]
*Yersinia pestis G*8786	Bv. Pestoides, isolated from Georgia	[5]
Yersinia pestis Rodent 24	Bv. Medievalis, isolated from rodent in Kurdistan	[6]
Yersinia pestis NCTC 10029	Bv. Antiqua, isolated from human, Nairobi, Kenya, 1958	The National Collection of Type Cultures (NCTC) for bacteria, UK
Yersinia pseudotuberculosis (B-1706)	Isolated from beaver (Germany)	Strain collection of the Bundeswehr Institute of Microbiology
Yersinia pseudotuberculosis (Y-714)	O:1a	Strain collection of the Bundeswehr Institute of Microbiology
Yersinia pseudotuberculosis (Y-715)	O:1a	Strain collection of the Bundeswehr Institute of Microbiology

Strain/phage/plasmid	Characteristics	Reference
Yersinia pseudotuberculosis (Y-716)	O:1a	Strain collection of the Bundeswehr Institute of Microbiology
Yersinia similis Y228^{T} (DSM18211)	O:6, isolated from rabbit	[7]
Yersinia wautersii (Y-428) (*Y. pseudotuberculosis* ('Korean group')	O:4a, isolated from badger	[8]
Yersinia enterocolitica (Y-929)	O:3, isolated from hare	Strain collection of the Bundeswehr Institute of Microbiology
Y. enterocolitica subsp. *palearctica* (DSM 13030)	O:3, human isolate	DSMZ-German Collection of Microorganisms and Cell Cultures
Phage L-413C (NC_004745)	Caudovirales; Myoviridae; Peduovirinae; Peduovirus: Morphotyp A1 - Serovar 2	[9]
Phage ΦA1122 (NC_004777)	Caudovirales; Podoviridae; Autographivirinae; Teseptimavirus; Morphotyp C1 - Serovar 1	[10]
pEGFP-C1	eGFP-Template	Takara Bio Europe SAS (Saint-Germain-en-Laye, France)-
mCherry-pBAD	mCherry-Template	[11]; Addgene plasmid #54630; http://n2t.net/addgene:54630
pASG-IBA 105*	*tet*-Promoter, *Twin-Strep-tag*, *lacP/Z*, *f1* ori, Amp^{R}, tetR, *colEI* ori,	IBA GmbH, Göttingen, Germany
pASG 105::TST::eGFP:: L-413C-RPB	Derivative of pASG-IBA 105, encodes fusion protein of Twin-Strep-tag, eGFP, gpH (RBP)	This work
pASG 105::TST::mCherry::L-413C-RPB	Derivative of pASG-IBA 105, encodes fusion protein of Twin-Strep-tag, mCherry, GpH (RBP)	This work
pASG 105::TST::eGFP:: ΦA1122-RPB	Derivative of pASG-IBA 105, encodes fusion protein of Twin-Strep-tag, eGFP, Gp17 (RBP)	This work
pASG 105::TST::mCherry:: ΦA1122-RPB	Derivative of pASG-IBA 105, encodes fusion protein of Twin-Strep-tag, mCherry, Gp17 (RBP)	This work

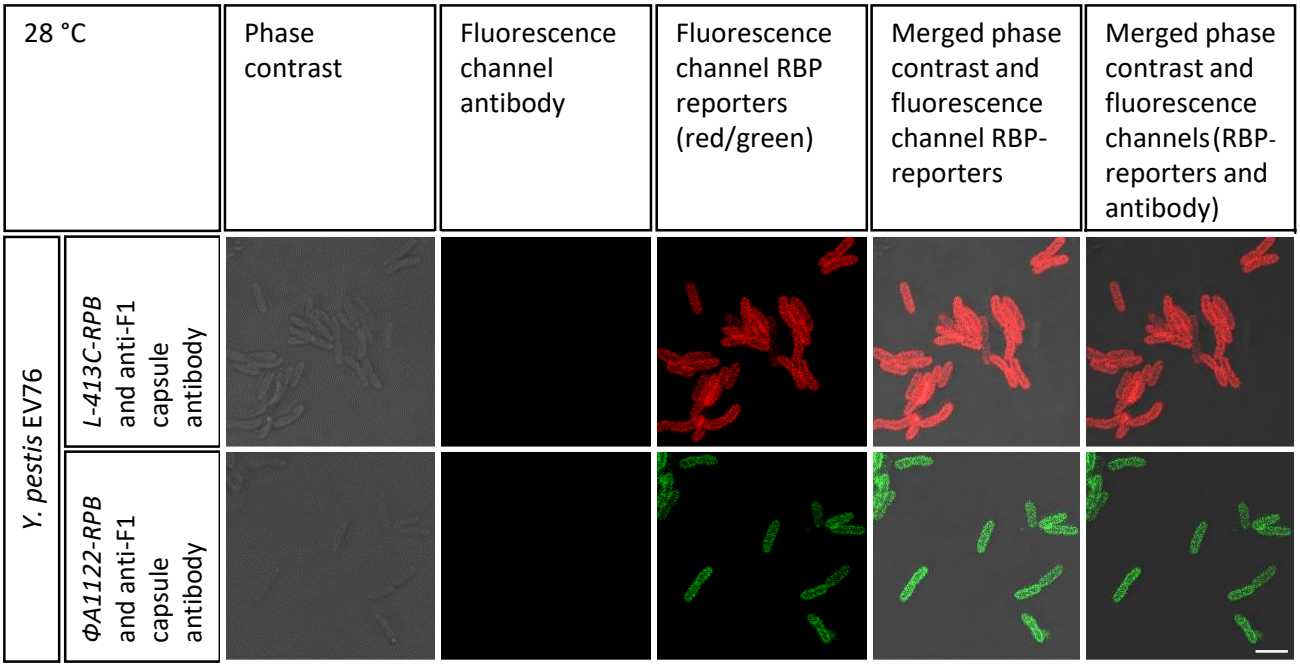
Supplementary Table S2: Oligonucleotide primer sequences used in this work.

Oligonucleotide	Sequence (5'-3')
L-413Cp19-RBP F	AAA CTC GAG TCT ACC AAA TTC AAA ACC GTT ATC ACC
L-413C-p19RBP R	AAA CGT ACG AAC CTG AGC AAC GTT GTA CCA

A1122p42-RBP F	AAA CTC GAG GCT AAC GTT ATC AAA ACC GTT CTG A
A1122p42-RBP R	AAA CGT ACG AAC ATC TTC AAC AGC GAT AG
eGFP forward	AGCGCGTCTCCAATGGTCGACGGTGAATTCGGCTGTACAGTGAGCAAGGGCGAGGAGC TGTTCAC
eGFP reverse	AGCGCGTCTCCTCCCCGTACGGCCCTGCAGACCCTCGAGCTTGTAGAGCTCGTCCATGCC GAGAG
mCherry forward	AGCGCGTCTCCAATGGTCGACGGTGAATTCGGCTGTACAGTTAGTAAAGGAGAAGAAA ATAACATGGC
mCherry reverse	AGCGCGTCTCCTCCCCGTACGGCCCTGCAGACCCTCGAGTTTGTATAGTTCATCCATGCC ACCAG



Supplementary Figure S1: Western blot of heterologously produced RBP fusion reporter proteins. Affinity purified proteins were subjected to SDS-PAGE, stained (Pierce stain) after transfer onto a nitrocellulose membrane and the TST epitope detected using a HRP-conjugated TST-antibody (StrepMAB, IBA GmbH, Göttingen, Germany). Expected sizes of RBP eGFP reporters: (a) L-413C RBP 130 kDa and (b) ΦA1122 RBP 93,5 kDa (indicated by arrows). Letters indicate size-positions of the protein size marker (SeeBlue Plus2 prestained, ThermoFisher Scientific, Darmstadt, Germany).



Supplementary Figure S2: Binding of RBP-reporters to growing cultures of *Y. pestis* EV76 cells at 28°C. In this representation, capsule formation was checked by incubation with a monoclonal anti-F1 capsule antigen antibody in combination with secondary antibody labelled with Alexa Fluor 488 for co-detection with L-413C-RBP-reporters or Alexa Fluor 647 for co-detection with ΦA1122-RPB-reporter, respectively. RBP binding to *Y. pestis* EV76 cells 6 h after inoculation at 28 °C is shown as individual representative micrographs for phage L-413C mCherry-RBP-reporter (red signals) or phage ΦA1122 eGFP-RBP-reporter (green signals) (as separate phase contrast, separate fluorescence or merged channels as indicated) (scale bar: 5 µm).

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