Supplementary Material

Potential of Bacteriophage Proteins as Recognition Molecules for Pathogen Detection

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Table S1 - Summary of several RBP-based systems available for the detection of bacterial pathogens.

Targeted pathogen	Phage RBP	Sample	Detection system	Detection limit	Response time	Ref.
Campylobacter	gp47*	Bacterial suspension	RBP-based agglutination assay, immunostaining, and fluorescence microscopy	Not reported	Not reported	(1)
	gp48*	Milk and chicken broth	RBP-based MS with TaqMan real-time PCR assay	10^2 CFU/mL	3 h	(2)
	gp48	Bacterial suspension	RBP-based MS, SPR and fluorescence microscope	10^2 CFU/mL	Not reported	(3)
	gp48	Bacterial suspension	RBP-based microresonator array platform	Single-cell limit	Not reported	(4)
Salmonella	gp37-gp38 complex	Bacterial suspension and spiked chicken, milk, chocolate milk and infant formula	RBP-based MS and ELLTA	1-10 CFU/mL	45 min	(5)
	RBP from phage P22	Bacterial suspension	RBP-NAEBs detection probes with SERS biosensor	Single-cell limit	Not reported	(6)
	RBPs from phage P22	Bacterial suspension	RBP-based SPR biosensor, fluorescence microscopy and scanning electron microscopy (SEM)	10^3 CFU/mL	Not reported	(7)
	RBPs from phages 9NA and P22	Bacterial suspension	RBP-based ELITA assay, MALTI-MS and flow cytometry	Not reported	Not reported	(8)
	Det7T	Bacterial suspension and spiked apple juice	RBP-based SPR biosensor	$5 \times 10^4 – 5 \times 10^7$ CFU/mL	20 min	(9)
Escherichia coli	gp37	Bacterial suspension	RBP-based LPG device	Not reported	Not reported	(10)
	gp37	Bacterial suspension	RBP-based LPG device	Not reported	1.5 h	(11)
	gp37	Bacterial suspension	Label-free RBP-based microwave sensors	Not reported	2 h	(12)
	gp37	Bacterial suspension	LPG-based device nano-coated with Tantalum oxide (TaO_X) thin layer	Not reported	30 min	(13)
	gp37	Bacterial suspension	Low-voltage field-emission scanning electron microscopy (LV-FESEM), energy-dispersive spectroscopy (EDS) and ELISA	Not reported	Not reported	(14)
	J	Bacterial suspension	RBP-based SPR	2×10^4 CFU/mL	20 min	(15)

Acinetobacter baumannii	TF2 and TF6	Bacterial suspension	RBP-based MS, MALDI–MS	10^4-10^5 cells/mL	10 min	(16)
Shigella flexneri	RBP from phage SF6	Bacterial suspension	RBP-based ELITA and fluorescence spectroscopy	10^3 CFU/mL	Not reported	(17)
Pseudomonas aeruginosa	P069	Bacterial suspension and spiked human urine, glucose injection and rat serum	RBP-based MS with bioluminescence and fluorescence spectroscopy	10^2 CFU mL	Not reported	(18)
	P069	Bacterial suspension and spiked milk, human urine, and physiological saline	Fluorescence microscopy and spectrofluorometry	2×10^3 CFU/mL	100 min	(19)
Yersinia pestis	gpH and gp17	Bacterial suspension	Fluorescence microscopy	Not reported	Not reported	(20)
Bacillus anthracis	RBPs from phages γ, Wip1, AP50c and λBa03	Bacterial suspension	Fluorescence microscopy	Not reported	10 min	(21)
Enterococcus and *Staphylococcus*	gp109 and gp18	Bacterial suspension and spiked blood	Epifluorescence microscopy and spectrofluorometry	10^8 CFU/mL	1.5 h	(22)
	gp109 and gp18	Bacterial suspension	RBP-based MS with MR Platform	10 CFU/mL	2 h	(23)
Klebsiella pneumoniae	gp86	Bacterial suspension	Epifluorescence microscopy and spectrofluorometry	10^8 CFU/mL	1.5 h	(24)

*gp47 and gp48 correspond to the same protein (1,25) but for information purposes are described herein as in the original papers. PCR - Polymerase chain reaction; SPR - Surface plasmon resonance; SERS - Surface-enhanced Raman scattering; NAEBs – Nanoaggregate embedded beads; LPG - Long period grating; MS -Magnetic separation; ELISA -Enzyme-linked immunosorbent assay; ELITA- ELISA-like tail spike absorption assay; ELLTA – Enzyme-linked long tail fibre assay; MR - magnetoresistive.

Table S2 - Summary of several phage endolysin CBD-based systems available for the detection of bacterial pathogens.

Targeted pathogen	Phage CBD	Sample	Detection system	Detection limit	Response time	Ref.
Listeria monocytogenes	CBD118 (Ply118) and CBD500 (Ply500)	Infected PtK2 epithelial cells	Fluorescence microscopy and CBD-based SPR	Not reported	Not reported	(26)
	CBD118 (Ply118) and CBD500 (Ply500)	Meat, poultry, fish, dairy products	CBD-MS	0.1 CFU/g	48 h	(27)
	CBD118 (Ply118) and CBD500 (Ply500)	Spiked raw milk	CBD-based MS, Culture-Based methods, and Real-Time PCR	10^2 - 10^3 CFU/mL	Not reported	(28)
	CBDP40 (PlyP40), CBDP35 (PlyP35), CBD500 (Ply500), CBDP35 (PlyP35), CBD025 (PlyP025)	Spiked milk and cheese	CBD-based MS, fluorescence microscopy	Not reported	At least 24 h	(29)
	CBD500 (Ply500)	Cell suspension and spiked milk	CBD-based electrochemical impedance biosensor	1.1 x 10^4 CFU/mL (suspension) 1.1 x 10^5 CFU/mL (milk)	Not reported	(30)
Listeria spp.	CBD118 (Ply118) and CBD500 (Ply500)	Cell suspension and 9 different food items	CBD-based MS and bioluminescent reporter phage (spectrophotometry)	10^2 CFU/mL (suspension) 0.1-1 CFU/g (food)	22 h	(31)
Bacillus anthracis	CBD of PlyG endolysin	Encapsulated cells in suspension and spores	CBD-based Membrane direct blot assay	10^3 CFU/mL	3 h	(32)
	Putative binding motifs of PlyG endolysin (PlyP1-P6*)*	Spiked plasma	QD based-Fluorometric analysis and Fluorescence microscopy	Not reported (single bacterium for PlyP3 peptide)	Not reported	(33)
Bacillus cereus	CBD of PBC1 endolysin	Spiked cooked rice	SPR-based detection method	10^2 CFU/mL (suspension) 10^3 CFU/mL (food)	Not reported	(34)
	CBD of endolysin LysB4	Cell suspension	Nitrocellulose-based lateral flow assay	10^4 CFU/mL	20 min	(35)
	CBD of PBC1 endolysin	Cell suspension and blood	Bioluminescent assay (spectrophotometry) with CBD-based MS	10 CFU/mL (suspension) 10^3 CFU/mL (blood)	At least 30 min	(36)

	CBD and SBD of LysPBC2	Cell suspension and spores	Fluorescence microscopy and spectrometry	Not reported	Not reported	(37)
Clostridium spp.	CBD of endolysin CTP1L	Cheese	GFP based protein - Fluorescence microscopy	3 spores/g	Not reported	(38)
Mycobacterium avium subsp. *paratuberculosis*	CBD of gp10 endolysin	Spiked milk	Pre-concentration by CBD-IMS and PCR	2.4 x 10^1 CFU/mL	24 h	(39)
Paenibacillus larvae	CBD of PlyPl23 endolysin	Homogenized honeybee larvae	Fluorescence microscopy	Not reported	Not reported	(40)
S. aureus, Bacillus anthracis-Sterne and *Listeria innocua*	lysostaphin CBD, AmiBA2446 CBD and Ply500 CBD	Cell suspension	CBD-based ELISA sandwich coupled with spectrophotometric assay and qPCR	10^3 CFU/mL (*S. aureus* and *B. anthracis*), 10^4 CFU/mL (*L. innocua*); qPCR: 2 CFU/mL (*S. aureus*), 3 CFU/mL (*B. anthracis*), 6 CFU/mL *L. innocua*)	Not reported	(41)
Staphylococcus aureus	CBD of endolysin PlyV12	Spiked milk	IMS and EL-CBD	4 x 10^3 CFU/mL	1.5 h	(42)
		Buffer and spiked serum and milk	CBD-based MS and spectrophotometry with fluorescent Amplex red/hydrogen peroxide-based assay	78 CFU/mL (buffer) 525 CFU/mL (milk) 348 CFU/mL (serum)	50 min	(43)
	Full-length LysK and truncated LysK	Cell suspension	Immobilization on silicon wafers, XPS and fluorescence microscopy	5 x 10^3 CFU/mL	Not reported	(44)
Staphylococcus spp.	AMICBD of E-LM12 endolysin	Cell suspension and spiked blood	AMICBD -based flow cytometer assay	1-5 CFU/mL	18 h	(45)
Methicillin-resistant *Staphylococcus aureus*	CBD from the P108 phage endolysin	Cell suspension	CBD-based lateral flow sandwich assay	6.6 × 10^2 - 6.6 × 10^7 CFU/mL	10 min	(46)
		physiological saline injection, lake water and human urine	CBD-MS and CBD-based flow cytometry assay	1.5 × 10^2 - 1.5 × 10^6 CFU/mL	Not reported	(47)

PCR - Polymerase chain reaction; IMS - Immunomagnetic separation; MS -Magnetic separation; EL-Enzyme linked; ELISA - Enzyme-linked immunosorbent assay; XPS - X-ray photoelectron spectroscopy; QD - Quantum-dots

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