Supplementary Data

Identification and characterization of phage protein and its activity against two strains of multidrug-resistant *Pseudomonas aeruginosa*

Fairoz Al-Wrafy[1,2*], Ewa Brzozowska[2*], Sabina Górska[2], Marek Drab[2], Magdalena Strus[3], Andrzej Gamian[2]

[1] Department of Applied Microbiology, Faculty of Applied Science, Taiz University, 6350 Taiz, Yemen.

[2] Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, 53-114 Wrocław, Poland.

[3] Department of Microbiology, Medical College, Jagiellonian University, 31-007 Krakow, Poland.

[*] Correspondence:

Fairoz Al-Wrafy: f_alwrafy@yahoo.com

Ewa Brzozowska: ewa.brzozowska@hirszfeld.pl

Antimicrobial agent	Disk content	Zone diameter, nearest whole [mm]			
		PAR21		PAR50	
Piperacillin	100 μg	28	S	14	R
Piperacillin- tazobactam	100/10 μg	28	S	25	S
Ticarcillin-clavulanic acid	75/10 μg	19	I	14	R
Ceftazidime	30 μg	27	S	25	S
Cefepime	30 μg	18	S	17	S
Cefotaxime	30 μg	20	I	22	I
Ceftriaxone	30 μg	< 13	R	< 13	R
Imipenem	10 μg	24	S	30	S
Ciprofloxacin	5 μg	32	S	35	S
Gentamicin	10 μg	17	S	<12	R
Amikacin	30 μg	20	S	14	R
Tobramycin	10 μg	18	S	<12	R
Netilmicin	30 μg	20	S	20	S

Table S1. Diameters of inhibition zones of different antibiotics against *P. aeruginosa* PAR21 and PAR50. R. Resistant, I. Intermediate and S. Susceptible

Antimicrobial agent	Disk content	Zone diameter, nearest whole [mm]		
		R	I	S
Piperacillin	100 μg	≤ 14	15-20	≥21
Piperacillin- tazobactam	100/10 μg	≤ 14	15-20	≥21
Ticarcillin-clavulanic acid	75/10 μg	≤ 15	16-23	≥24
Ceftazidime	30 μg	≤ 14	15-17	≥ 18
Cefepime	30 μg	≤ 14	15-17	≥ 18
Cefotaxime	30 μg	≤ 14	15-22	≥ 23
Ceftriaxone	30 μg	≤13	14-20	≥ 21
Imipenm	10 μg	≤ 13	14-15	≥16
Ciprofloxacin	5 μg	≤15	16-20	≥21
Gentamicin	10 μg	≤12	13-14	≥15
Amikacin	30 μg	≤ 14	15-16	≥17
Tobramycin	10 μg	≤12	13-14	≥15
Netilmicin	30 μg	≤12	13-14	≥15

Table S2. The inhibition zone diameter for each antibiotic according to the criteria published by the Clinical and Laboratory Standards Institute (CLSI)[1] . **R**. Resistant, **I**. Intermediate and **S**. Susceptible

Figure S1. Spot assay using 10 μl phage sample applied on a lawn of *P. aeruginosa* strains. Translucent clearance at the spot area was observed against PAR50, but not PAR21.

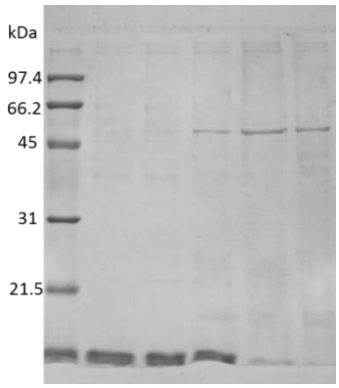

Figure S2. Full-length uncropped gel used in figure 1.

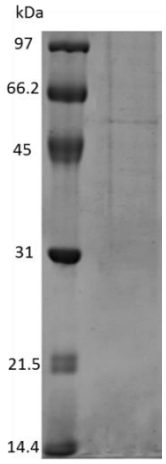

Figure S3. Full-length gel used in figure 2.

Figure S4. Identification of PA-PP1 protein using mass spectrometer LC-MS/MS. The protein sequence, matched peptides are shown in bold red.

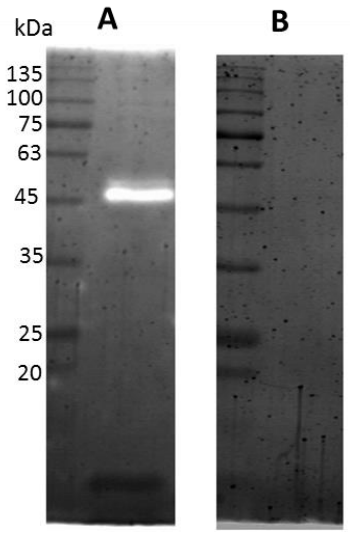

Figure S5. Full-length gel used in figure **3**

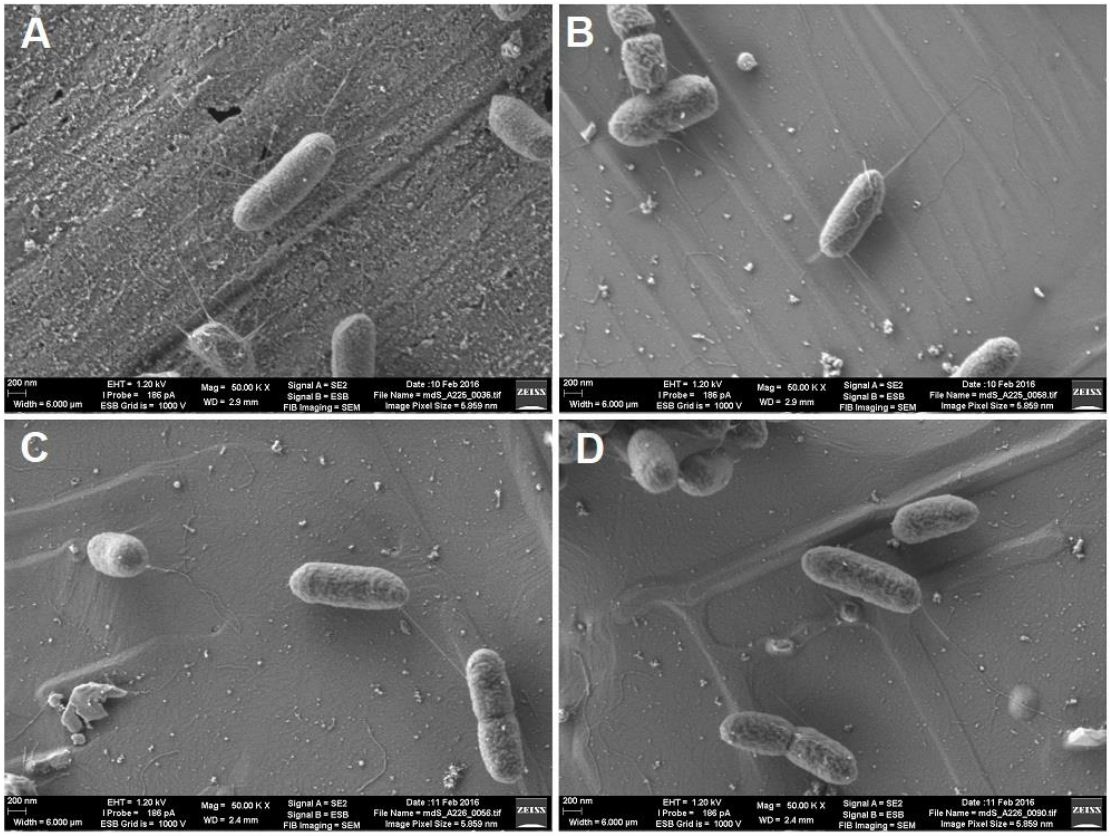

Figure S6. Low voltage scanning electron microscopy (LV-SEM) to determine the effects of PA-PP on *P. aeruginosa* PAR21. A and B represent intact cells not exposed to protein. C and D represent PAR21 cells exposed to PA-PP, which appear similar to the non-treated cells showing no defects.

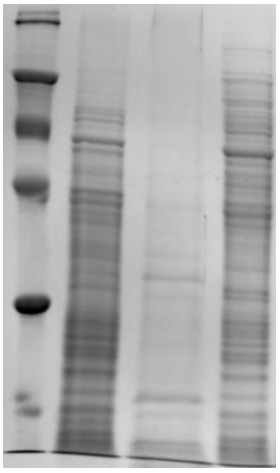

Figure S7. Full-length gel used in figure 8.

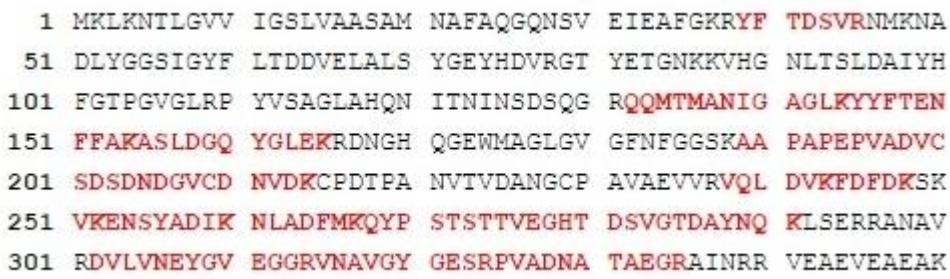

Figure S8. Identification of bacterial OM protein which was targeted by PA-PP protein mass spectrometer LC-MS/MS. The protein sequence obtained from the mass spectrometer LC-MS/MS, matched peptides are shown in bold red.

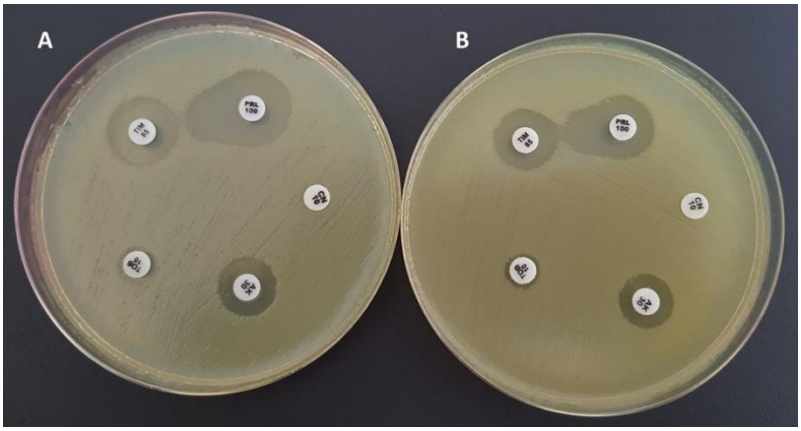

Figure S9. Synergistic activity of antibiotics and PA-PP. **A**. Antibiotics were applied to *P. aeruginosa* PAR50 treated with PA-PP protein. **B**. Antibiotics were applied to untreated *P. aeruginosa* PAR50.

1- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Informational supplement M100–S24, 24th ed. 34, 58–60. (Clinical and Laboratory Standards Institute, Wayne, PA. USA. 2014).