Potential control of potato soft rot disease by the obligate predators *Bdellovibrio* and like organisms

Daniel Youdkes, Yael Helman, Saul Burdman, Ofra Matan, Edouard Jurkevitch

Department of Plant Pathology and Microbiology, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, 7610001, Israel.

Supplementary material

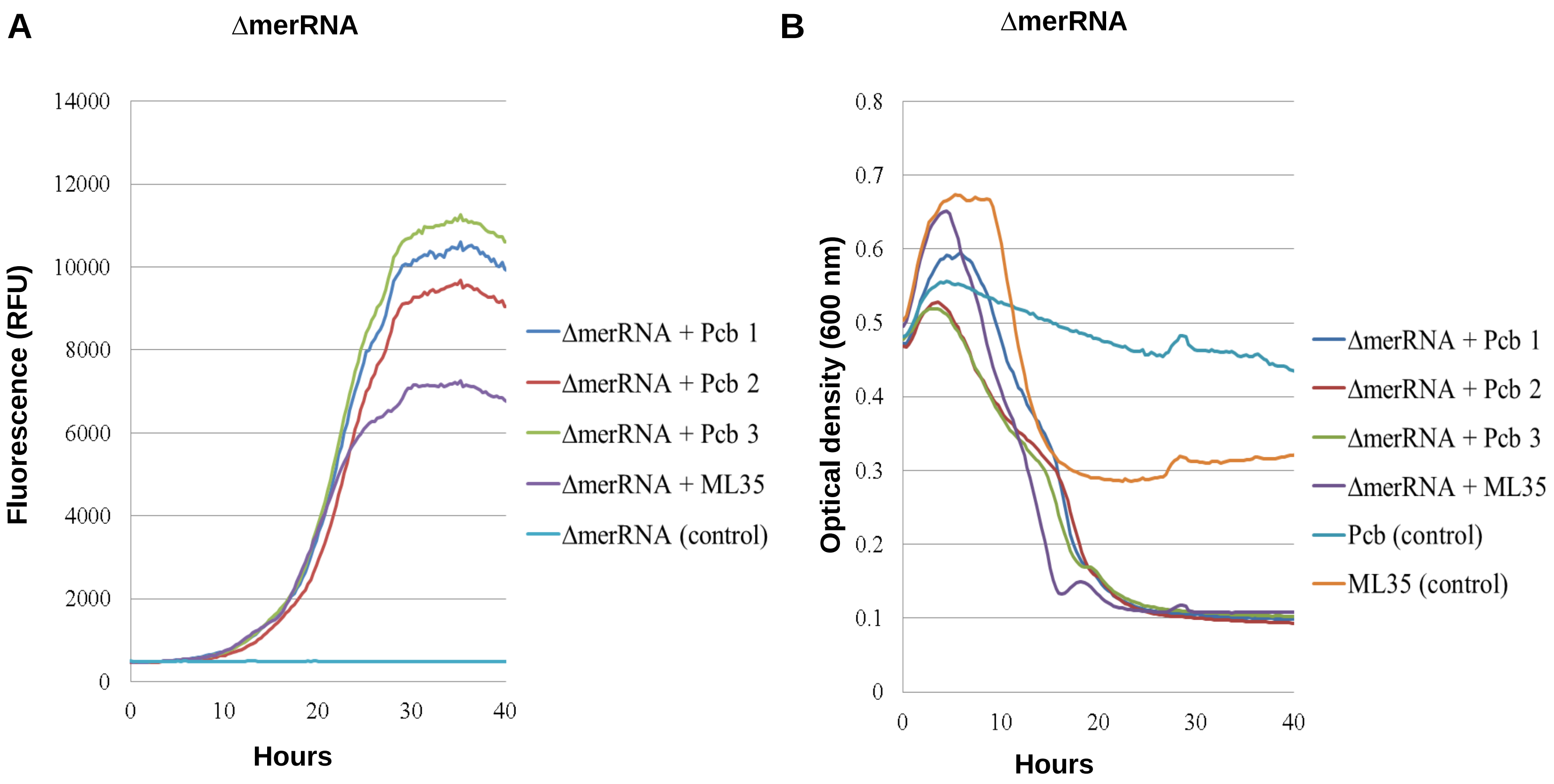


Figure S1. Growth of *B. bacteriovorus ΔmerRNA*-TdTomato and decay of the *Pectobacterium. carotovorum* subsp. *brasiliense* (Pcb) and *E. coli* ML35 prey populations. The predatory populations are tracked by fluorescence (relative fluorescence units, RFU)(A), the prey populations by optical density (OD)(B).

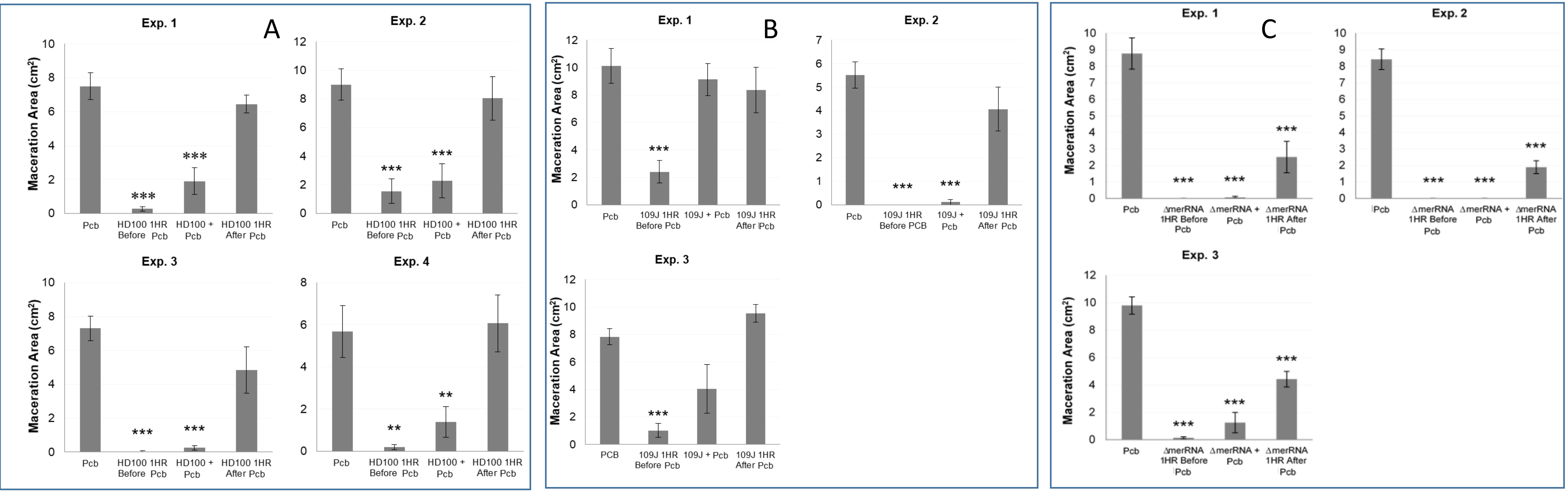


Figure S2: : The effect of *B. bacteriovorus* strains HD100 (A), 109J (B), and *ΔmerRNA* (Δ*mer*) (C), on the development of tissue maceration in potato slices, induced by the soft rot bacterium *P. carotovorum* subsp. *brasiliense* (Pcb). *B. bacteriovorus* (10 µl, 10^9 PFU.ml^{-1}) inoculated 60 min before, together with or 60 min after Pcb (10 µl, 10^6 CFU mL^{-1}). Error bars represent standard error of 10 replicates in each of three independent experiments. Asterisks indicate significant difference compared to control (Pcb) according to Student's t-test (*** = p<0.001; ** = p<0.01).

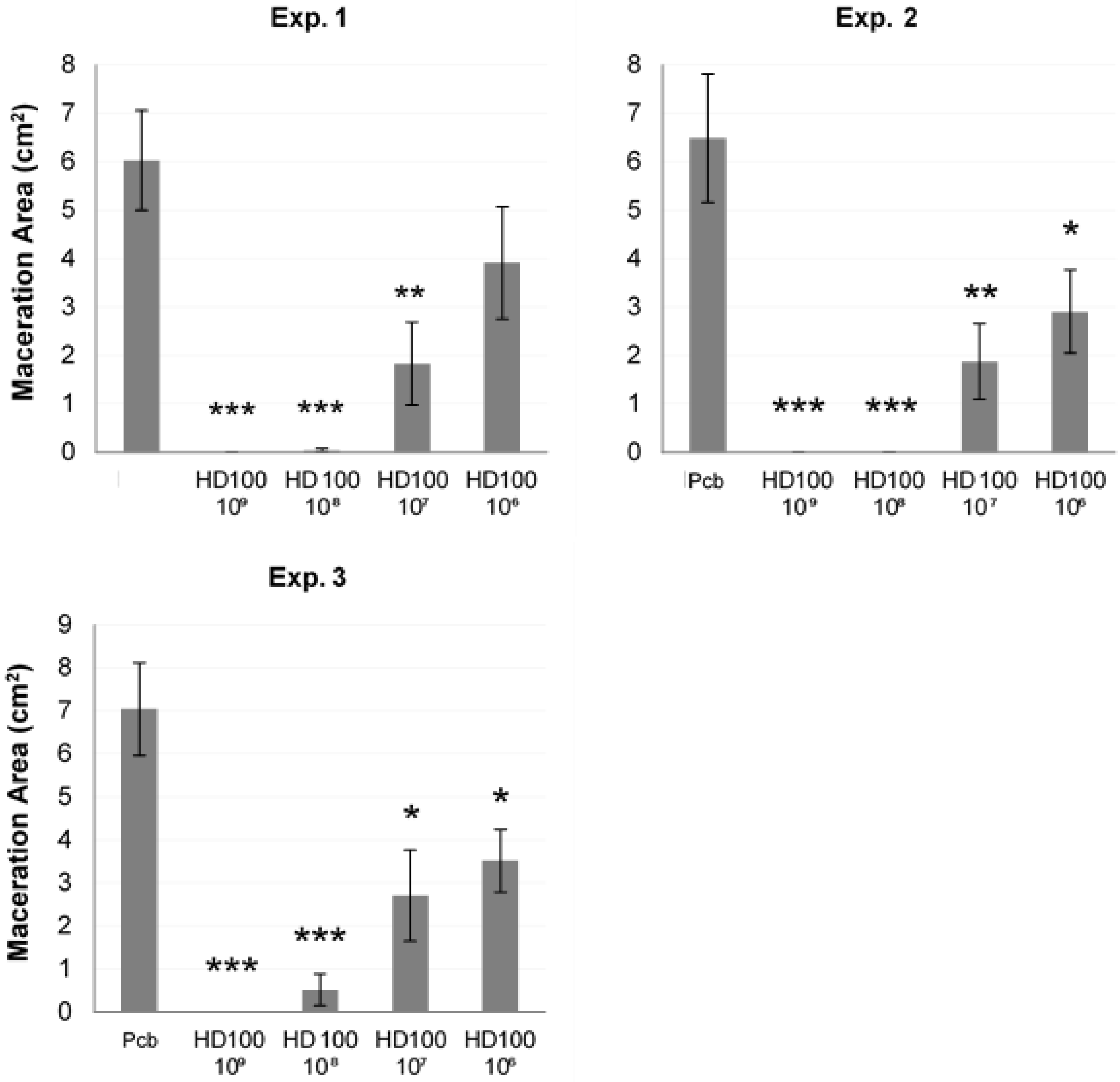


Figure S3. The effect of *B. bacteriovorus* strains HD100 inoculated at concentrations 10^6, 10^7, 10^8, and 10^9 PFU.ml^{-1} (10 µl) on the development of tissue maceration in potato slices, induced by the soft rot bacterium *P. carotovorum* subsp. *brasiliense* (Pcb) (10 µl, 10^6 CFU ml^{-1}) inoculated 60 min after the predator. Error bars represent standard error of 10 replicates in each of three independent experiments. Asterisks indicate significant difference compared to control (Pcb) according to Student's t-test (*** = p<0.001; ** = p<0.01; * = p<0.05).

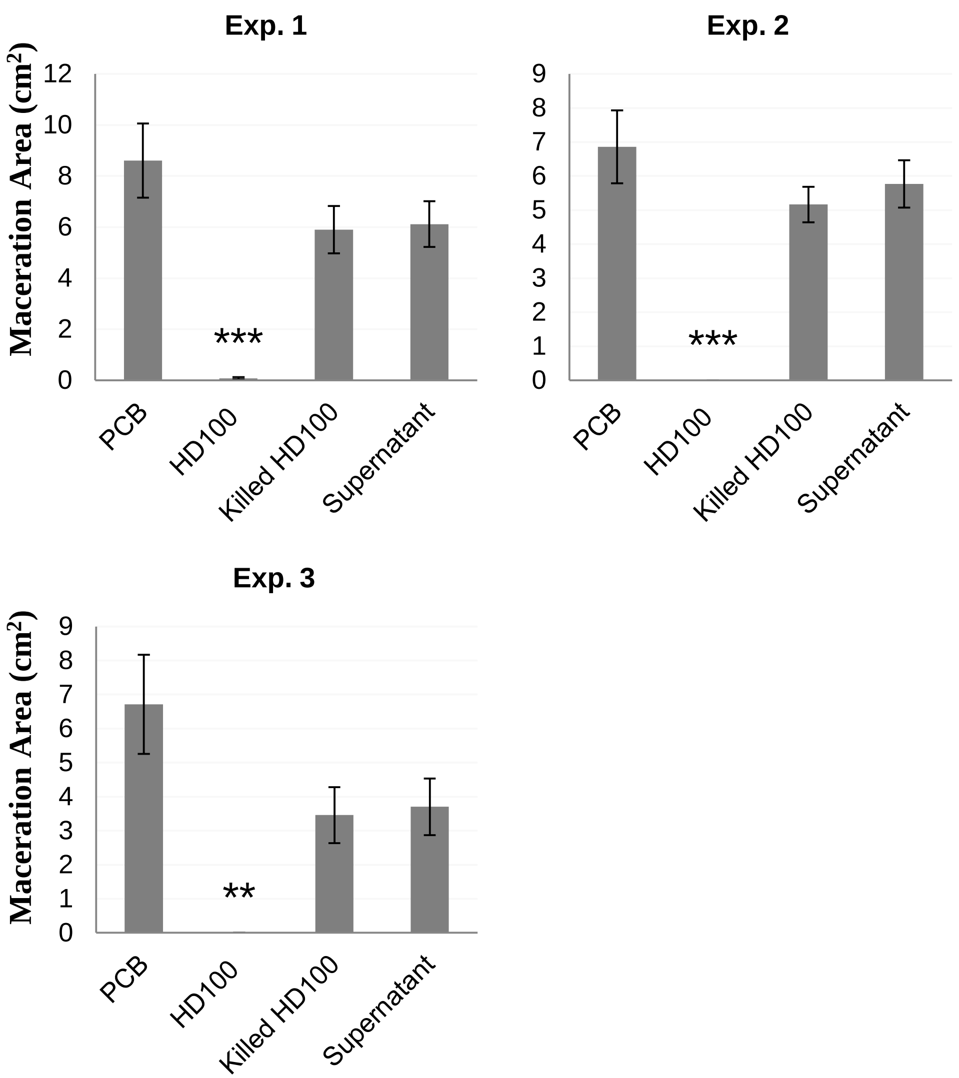
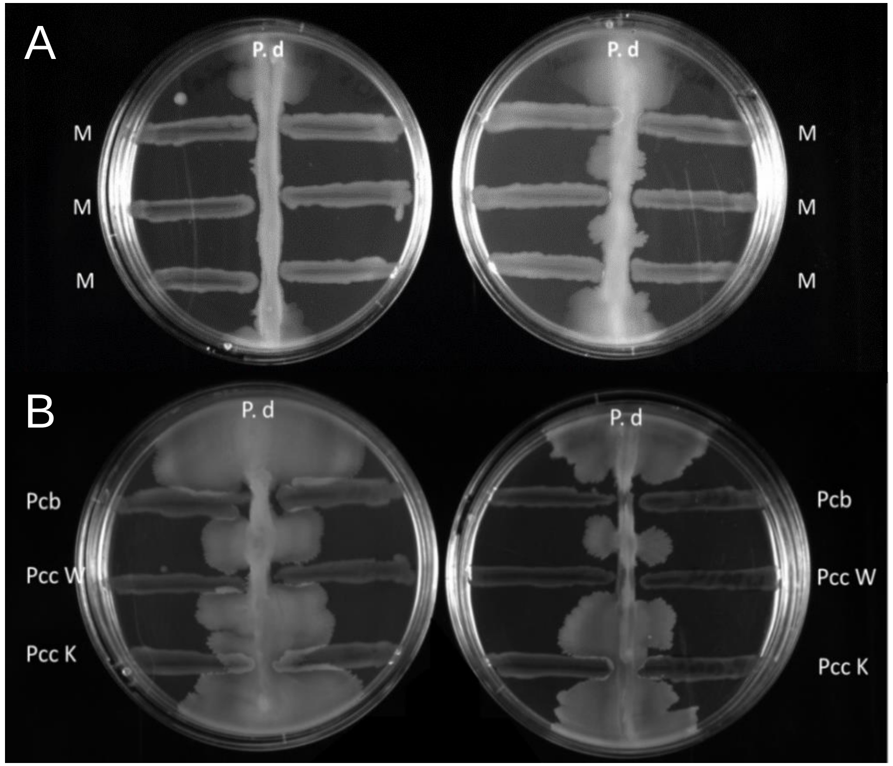
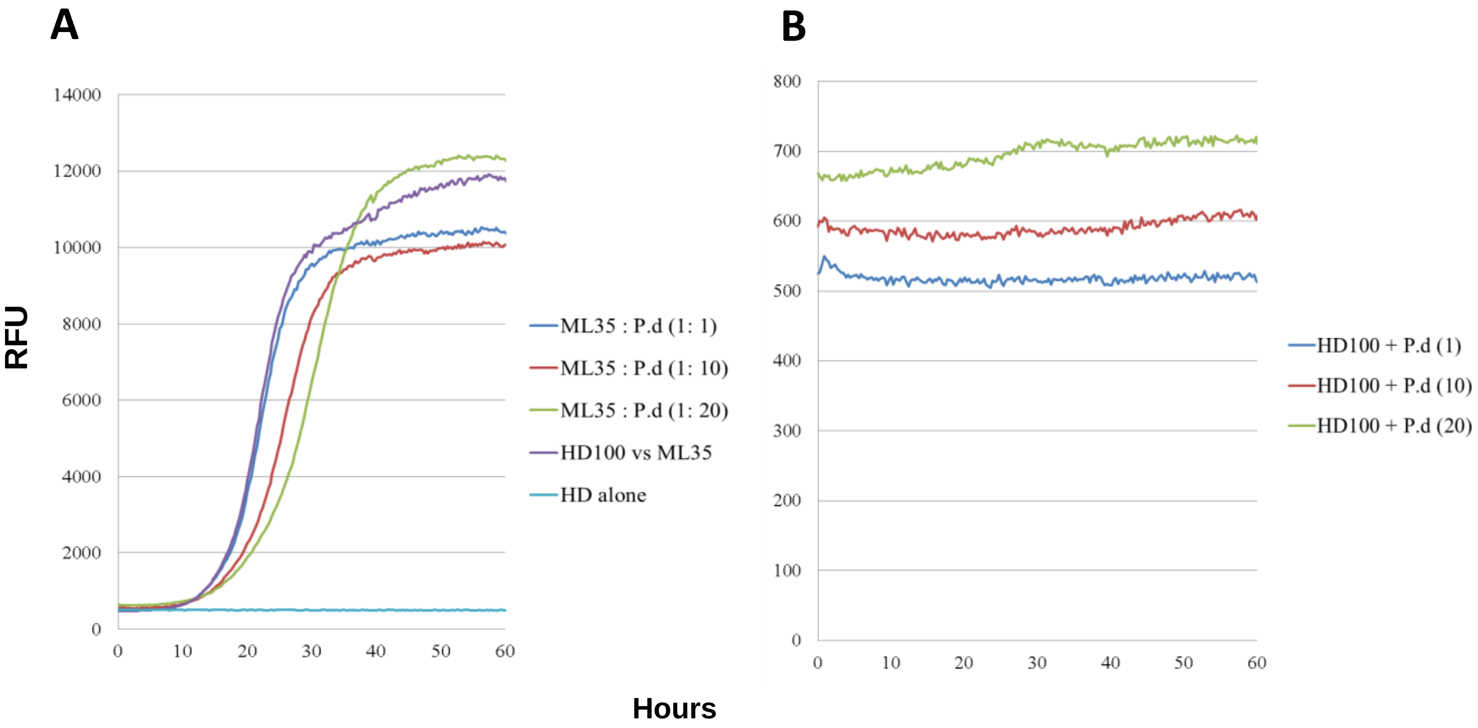


Figure S4: The effect of inoculating *B. bacteriovorus* HD100 (10 μl, 10^9 PFU.ml^{-1}) with live, heat-deactivated (killed) HD100, and with the supernatant of a *P. carotovorum* subsp. *brasilense* lytic culture, 60 minutes before *P. carotovorum* subsp*. brasiliense* (Pcb) (10 μl, 10^6 CFU mL^{-1}) on disease development in potato slices. Error bars represent standard error of 10 replicates in each of three independent experiments Asterisks indicate significant difference compared to control (Pcb) according to Student's t-test (*** = p<0.001; ** = p<0.01).





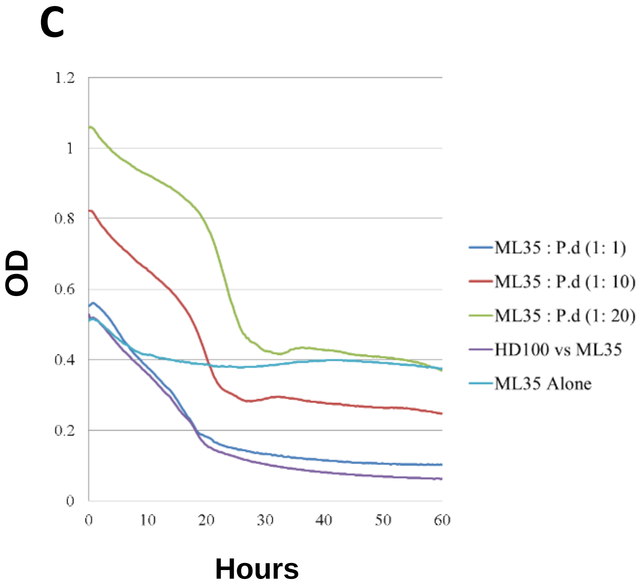


Figure S5. : Left panel. Streak assay testing the effect of *P. dendritiformis* (P. d)) on the growth of: A. *E. coli* ML35 (M), and B. *P. carotovorum* subsp. *brasiliense*, *P. carotovorum* subsp *caratovorum* WPP14 and KC24 (Pcb, Pcc W and Pcc K, respectively). Pcb was streaked on LB plates 48 prior to the tested bacteria. The plates were incubated for an additional 120 h. Right panel**.** Growth of *B. bacteriovorus* HD100 (HD100), and decay of the *E. coli* ML35 (ML35) prey in the presence of *P. dendritiformis* (P.d). The predatory populations and a P.d. only control were measured by fluorescence (relative fluorescence units. RFU)(A, B), the prey populations by optical density (OD) (C). The ratios of of *E. coli* ML35 to *P. dendritiformis* varied from 1:1, 1:10, to 1:20.

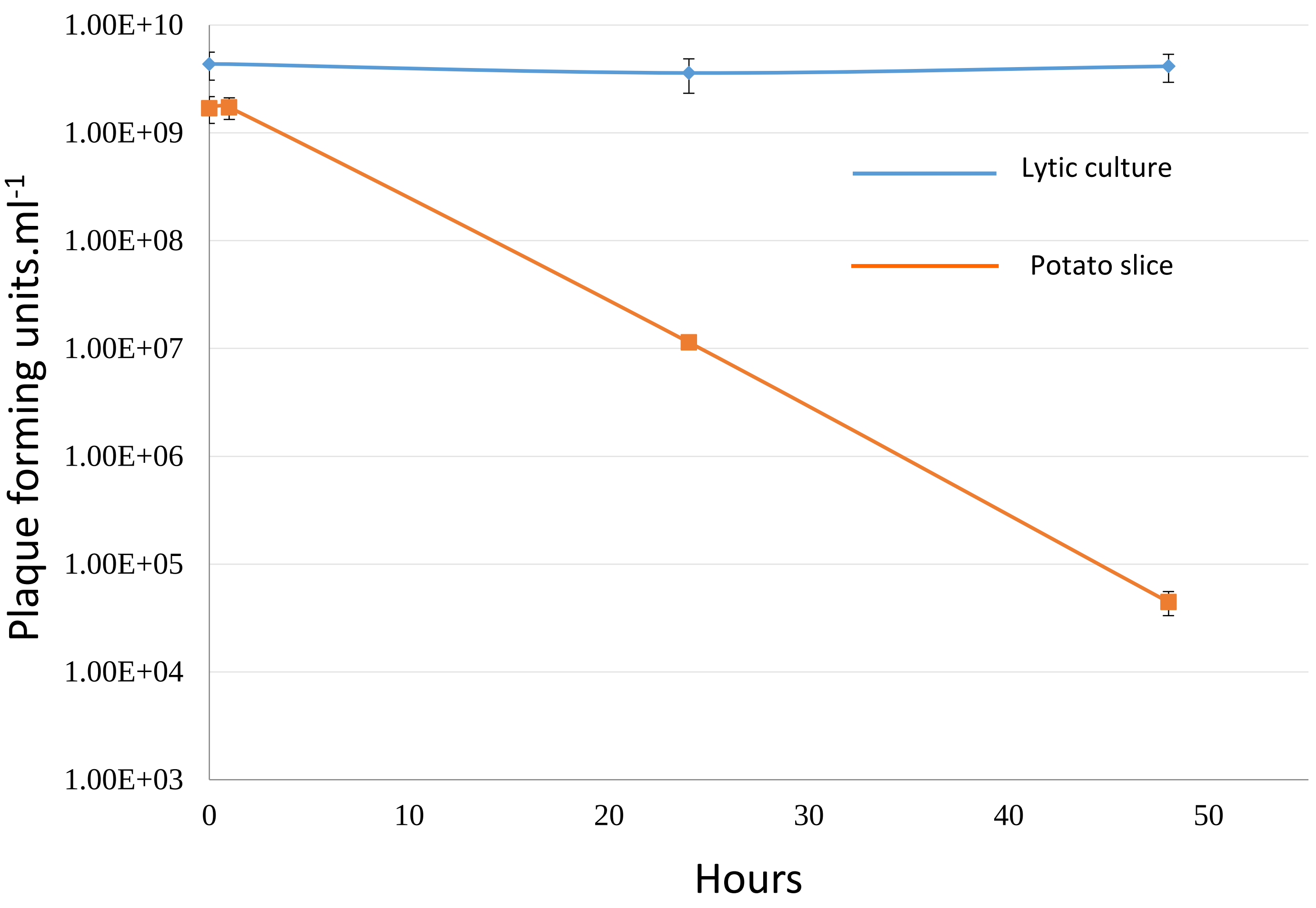
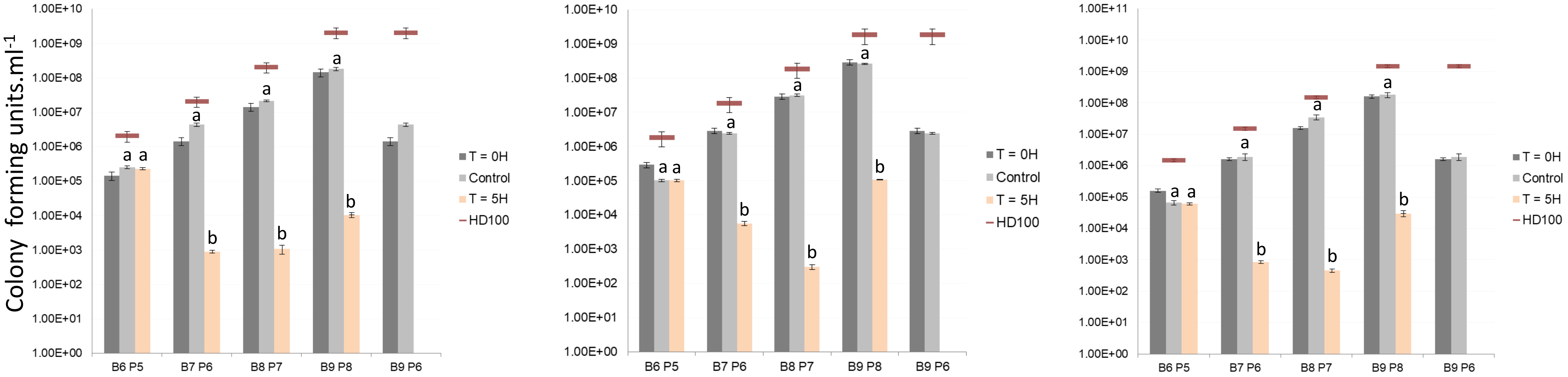


Figure S6. Survival of *B. bacteriovorus* HD100 inoculated on potato slices (10 µl, 10^9 PFU.ml^{-1}), and in lytic cuture, incubated for 0, 1, 24 and 48 hours at 28 °C. Error bars represent standard error of three replicates.

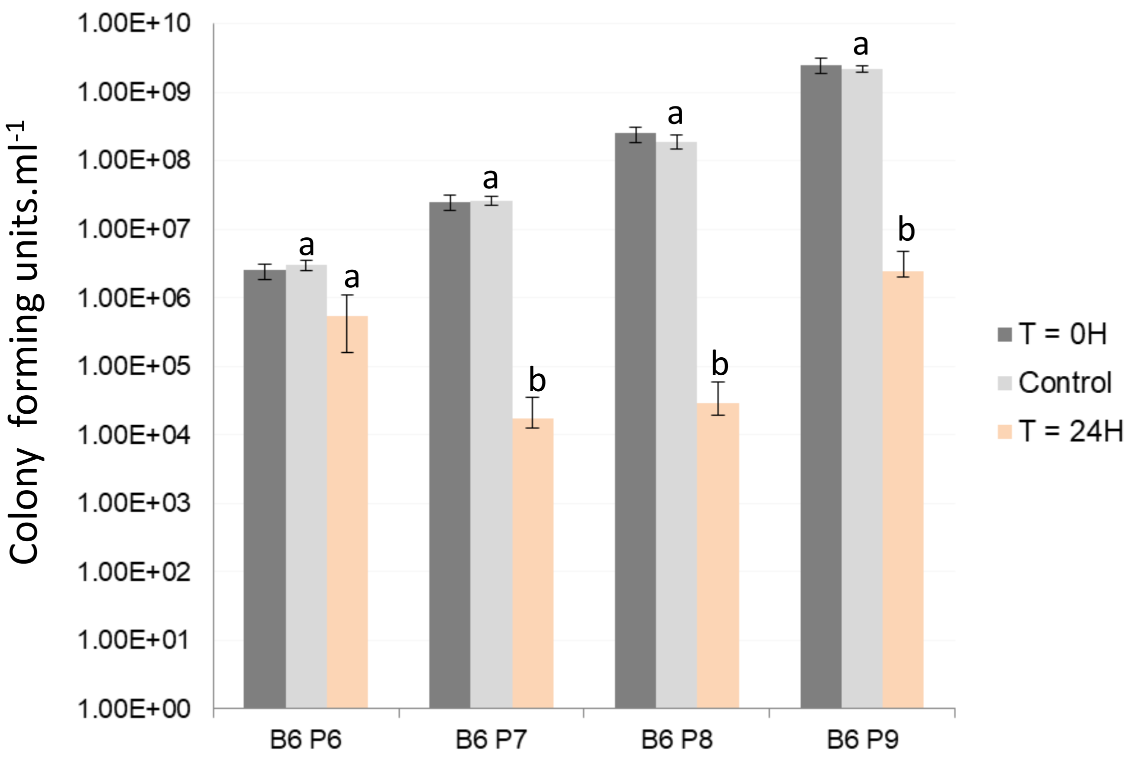


Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value
B9P8	**B8P7**	10	2.98317	3.35214	**0.0072***
B8P7	**B7P6**	4.4167	2.980842	1.48168	**0.5744**
B7P6	**B6P5**	-12.4167	2.983752	-4.16143	**0.0003***

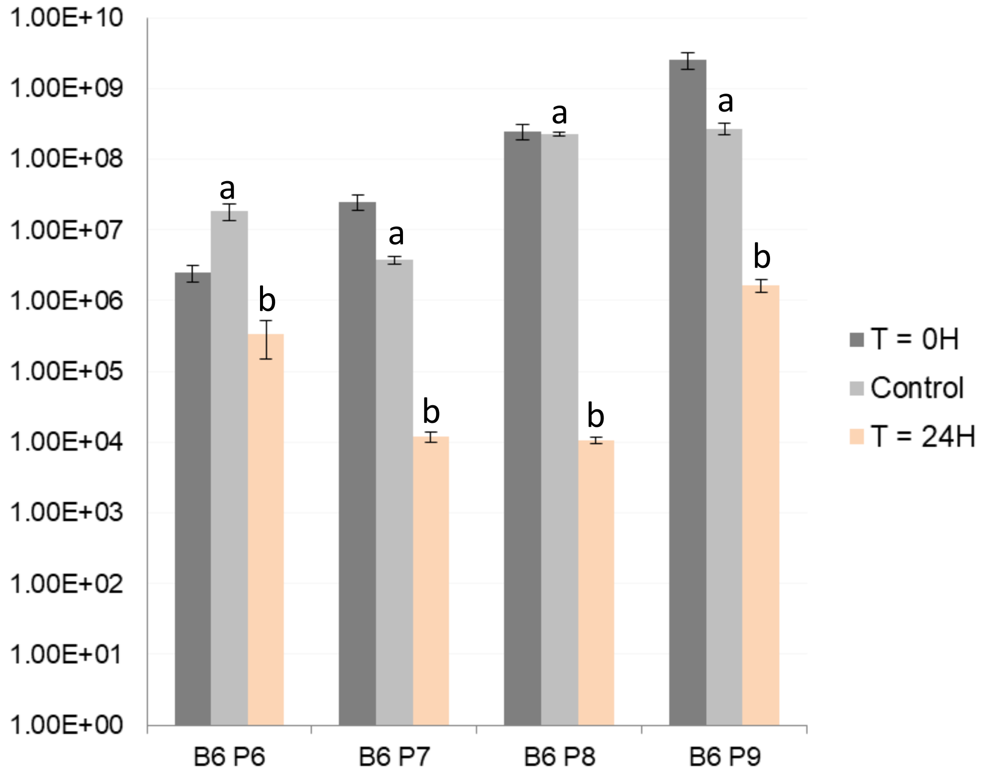
Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value
B9P8	**B8P7**	14.9333	3.20237	4.66321	**<.0001***
B7P6	**B6P5**	-14.9333	3.208466	-4.65435	**<.0001***
B8P7	**B7P6**	-14.9333	3.203446	-4.66165	**<.0001***

Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value
B9P8	**B8P7**	10.9333	3.188341	3.42916	**0.0034***
B8P7	**B7P6**	-10.8	3.182206	-3.39387	**0.0038***
B7P6	**B6P5**	-14.9333	3.18798	-4.68426	**<.0001***

Figure S7. Surviving *P. carotovorum* subsp. *brasiliense* prey (colony forming units.ml^{-1}) after predation by *B. bacteriovorus* HD100 in aHEPES at concentrations of: A. 10^6, 10^7, 10^8. and 10^9 PFU.ml^{-1} predators and 10^5, 10^6, 10^7 and 10^8 CFU.ml^{-1} prey. Prey concentration at the beginning of the experiment (dark grey bars), at the end of the experiment (5 h) without predator (light grey bars), or with predator (pink bars). Predator: prey ratio was kept at 10:1. Red horizontal bars are the concentration of the predator and the end of the experiment. B denotes predator, P denotes prey, the number denotes the exponent of the initial concentration. Error bars indicate standard error. Different letters differentiate ($p < 0.01$) between treatment with and without predator within each BP combination. Tables below each graph show statistics of differences between treatments with predators at different starting predator and prey concentrations (pink bars).



Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value
B6 P9	B6 P8	11.4042	2.942022	3.8763	0.0006*
B6 P8	B6 P7	5.3977	2.562662	2.1063	0.151
B6 P7	B6 P6	-8.3712	2.524876	-3.31549	0.0051*



Level	- Level	Score Mean Difference	Std Err Dif	Z	p-Value
B6 P9	B6 P8	14.9333	3.202011	4.66374	<.0001*
B6 P8	B6 P7	0.6667	3.195183	0.20865	0.9968
B6 P7	B6 P6	-9.8667	3.051603	-3.23327	0.0067*

Figure S8. Surviving *P. carotovorum* subsp. *brasiliense* (colony forming units.ml^{-1}) after predation by *B. bacteriovorus* HD100 in aHEPES at inititial concentrations of 10^6 PFU.ml^{-1}, predators and 10^6, 10^7, 10^8 and 10^9 CFU.ml^{-1} prey, 24 hours (T = 24H) after mixing predation and prey. B denotes predator, P denotes prey, the number denotes the exponent of the initial concentration. Error bars indicate standard error. Within each treatment (B_iP_j) bars not connected by the same letter are significantly different ($p < 0.01$). Tables below each graph show statistics of differences between treatments with prey at different starting concentrations.

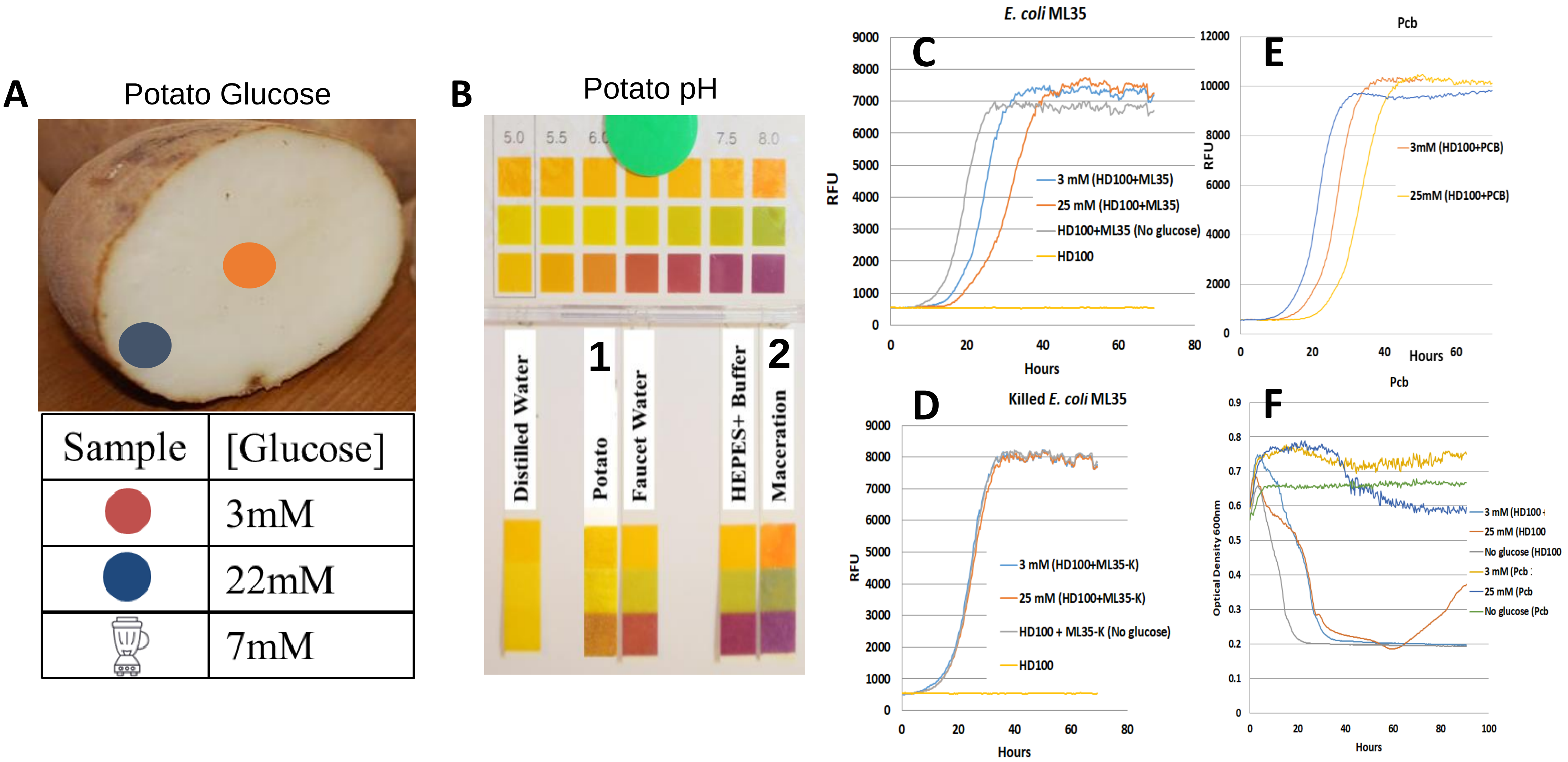


Figure S9. Left panel. A. Average glucose concentrations of three readings in mM from center (red), edge (blue), and blended potato (blender symbol). B. pH of potato slices before infection (1) and after infection (2). pH strips were directly inserted into the macerated tissue 48 hours after inoculation with *P. carotovorum* subsp. *brasiliense*. This measurement was carried out on 10 different potatoes, and the result presented is a representative result from one of those measurements. Right panel. Growth of *B. bacteriovorus* HD100 (HD100) on live (C) and heat-killed (D) *E. coli* ML35 (ML35), on *P. carotovorum* subsp. *brasilense* (Pcb) (E) , in the presence of 0, 3 and 25 mM glucose, measured by fluorescence (relative fluorescence units. RFU), and decay of the Pcb population measured by optical density (OD)(F).

Table S1. Predation of *B bacteriovorus* strains HD100, 109J, *ΔmerRNA* and *M. aeruginosavorus* ARL-13 upon the soft rot-causing *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb) 1692, *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) strain WPP14 and KC24, and *Dickeya solani*.

Predator/prey	Pcb. 1692	Pcc. WPP14	Pcc. KC24	*D. solani*
B. bacteriovorus HD100	✓	✓	✓	✓
B. bacteriovorus 109J	✓	✓	✓	✓
B. bacteriovorus ΔmerRNA	✓	✓	✓	✓
M. aeruginosavorus ARL13	✓	✓	✓	**x**

✓ indicates predation, assessed as a decrease in prey culture turbidity after overnight incubation in the presence of a predator, confirmed by phase contrast microscopy. 'X' indicates failure to predate.

Table S2. Average predation time based on OD measurements of the prey population (in hours). Prey: *P. carotovorum* subsp *brasiliense* (Pcb 1692), subsp *carotovorum* WPP14 and KC24 (Pcc WPP14, Pcc KC24), *Dickeya solani*, *E. coli* ML35, and *Pseudomonas aeruginosa*.

Predator/Prey	**Pcb 1692**	**Pcc WPP14**	**Pcc KC24**	***D. solani***	***E. coli* ML35**
B. bacteriovorus HD100	15	15	22	40	15
B. bacteriovorus	15	15	20	80	12
B. bacteriovorus	17	-	-	-	16
M. aeruginosavorus ARL-13	65	65	40	X	-

'**-**' indicates the combination of predator and prey was not tested, '**x**' indicates no predation observed. (n = 3)**.**

Table S3. Upper table: Number of macerated potato slices (out of 5) caused by different concentrations of *P. carotovorum* subsp. *brasiliense* (Pcb). Lower table: Number of macerated potato slices (out of 5) caused by different concentrations of *Dickeya solani.* Each bacterium was tested in four independent experiments (left column).

	Pcb concentration (CFU ml^{-1})				**Control**	
	10^7	**10^6**	**10^5**	**10^4**	Buffer	No treatment
1	5/5	3/5	0/5	0/5	0/5	0/5
2	5/5	5/5	2/5	3/5	0/5	0/5
3	5/5	4/5	4/5	1/5	0/5	0/5
4	5/5	4/5	1/5	1/5	0/5	0/5

	Treatment (CFU ml^{-1})				**Control**	
	10^7	**10^6**	**10^5**	**10^4**	Buffer	No treatment
1	4/5	3/5	0/5	0/5	0/5	0/5
2	3/5	0/5	0/5	0/5	0/5	0/5
3	3/5	1/5	0/5	0/5	0/5	0/5
4	3/5	0/5	0/5	0/5	0/5	0/5