Supplemental Material – **A quantitative analysis of *Lysobacter* predation**

Ivana Seccareccia,[1] *Christian Kost,*[2] *and Markus Nett*[1*]

[1]Junior Research Group "Secondary Metabolism of Predatory Bacteria", Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, 07745 Jena, Germany

[2]Research group "Experimental Ecology and Evolution", Max Planck Institute for Chemical Ecology, 07745 Jena, Germany

*Corresponding author. Mailing address: Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Adolf-Reichwein-Str. 23, 07745 Jena, Germany. Phone: 49 3641 532 1297. Fax: 49 3641 532 0811. E-mail: markus.nett@hki-jena.de

Table of Contents

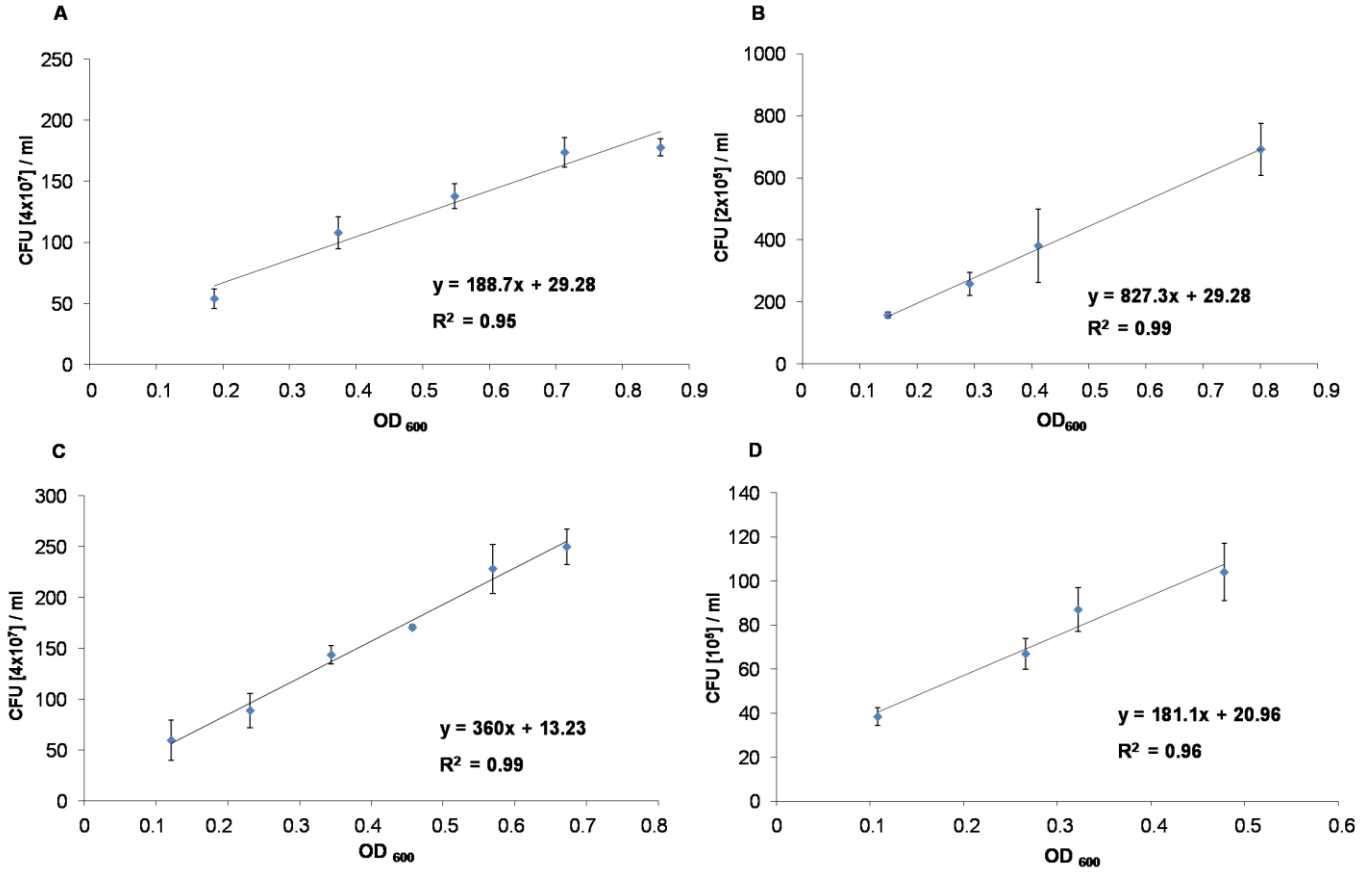

Figure S1. OD_{600}-CFU plot for *Lysobacter capsici* DSM 19286 (A), *L. enzymogenes* DSM 2043 (B), *L. oryzae* DSM 21044 (C), and *Myxococcus fulvus* ST035975 (D). Linear regression analysis was used for estimating the relationship between optical density at 600 nm (OD_{600}) and CFU/ml. Prediction was made within the range of values in the dataset used for model-fitting.

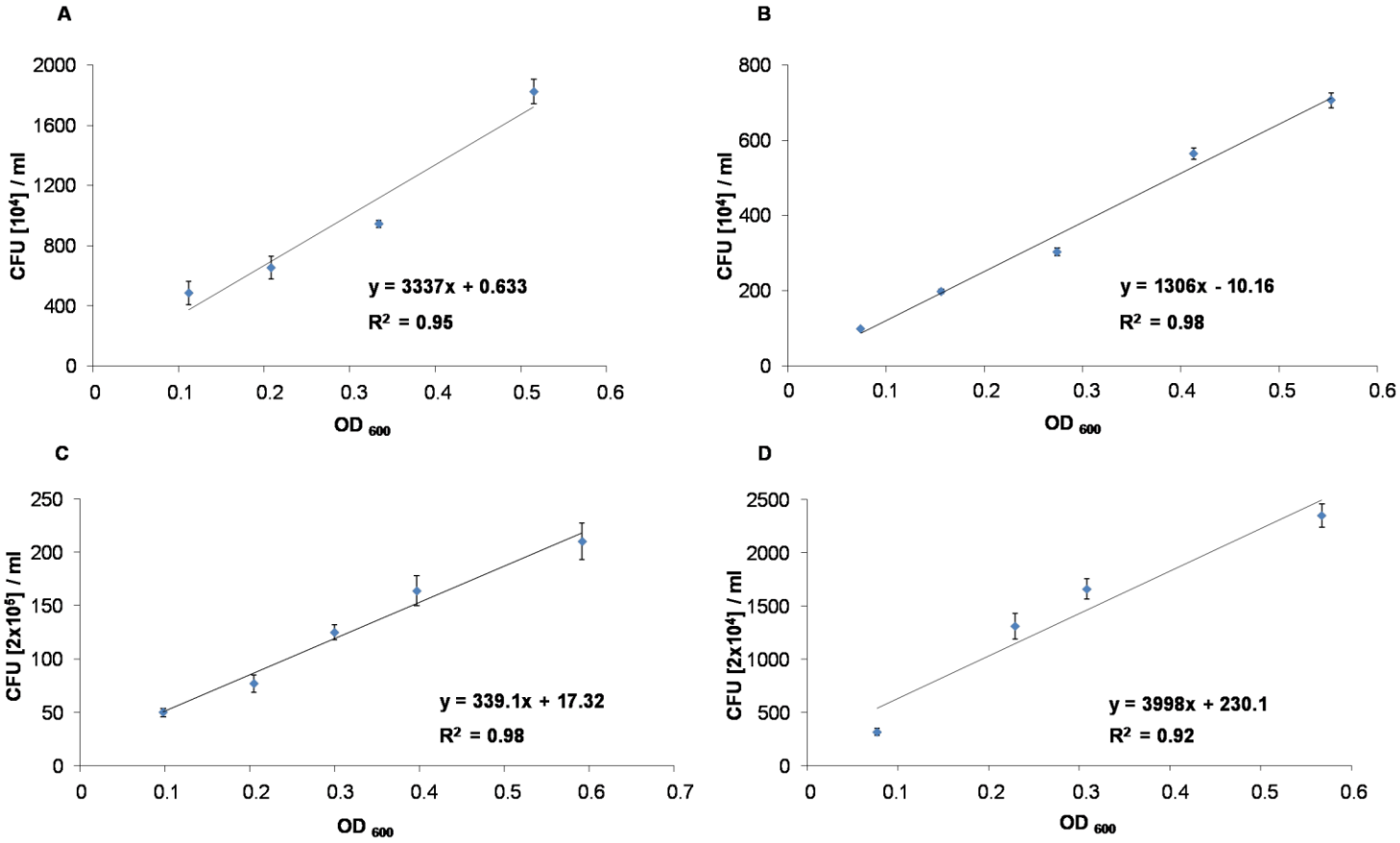

Figure S2. OD_{600}-CFU plot for *Agrobacterium tumefaciens* DSM 5172 (A), *Bacillus subtilis* DSM 347 (B), *Chromobacterium pseudoviolaceum* DSM 23279 (C), and *Escherichia coli* DSM 18039 (D). Linear regression analysis was used for estimating the relationship between optical density at 600 nm (OD_{600}) and CFU/ml. Prediction was made within the range of values in the dataset used for model-fitting.

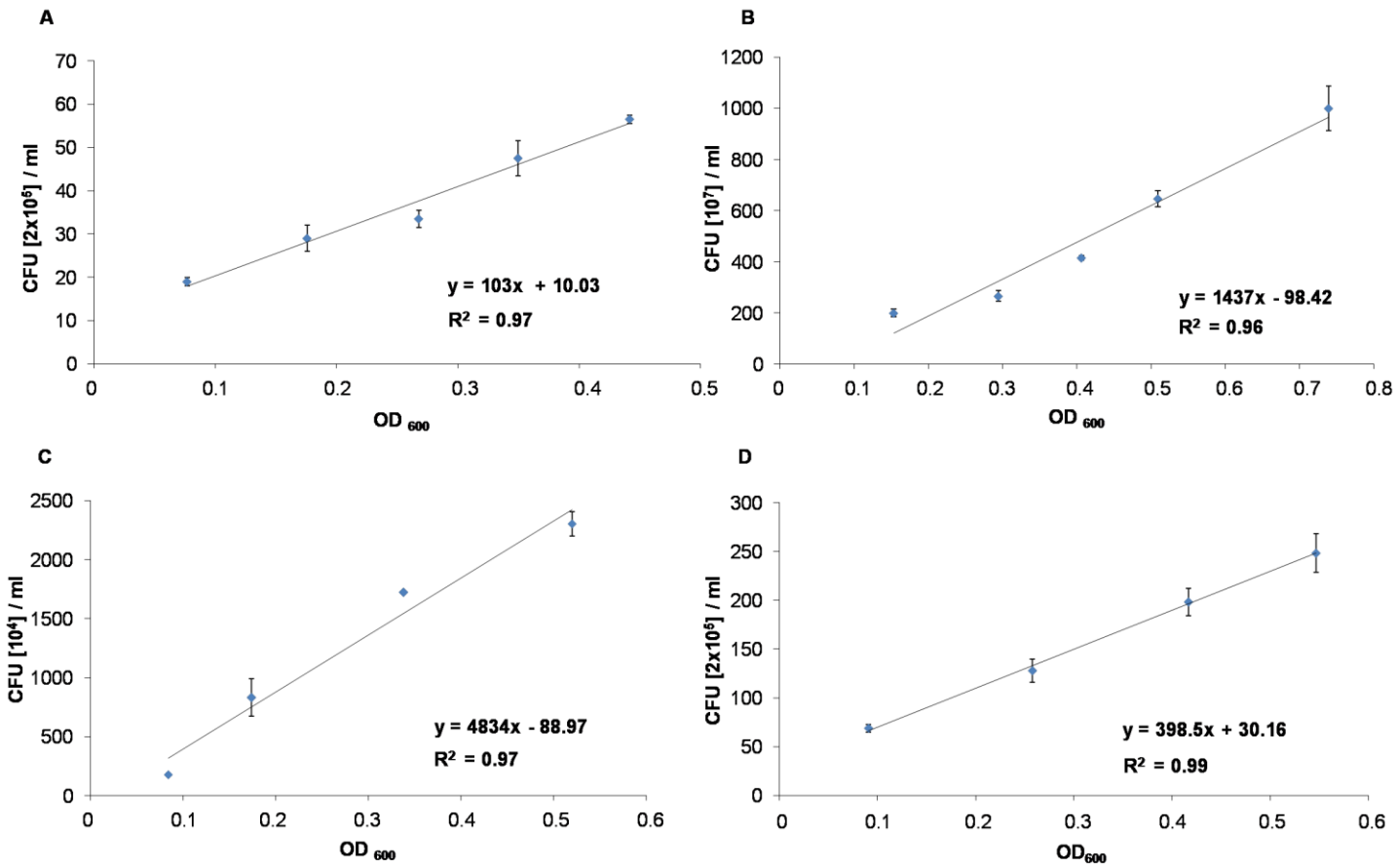

Figure S3. OD_{600}-CFU plot for *Lactococcus lactis* DSM 20069 (A), *Pseudomonas fluorescens* DSM 11532 (B), *Ralstonia solanacearum* GMI1000 (C), and *Rhodococcus rhodochrous* DSM 43334 (D). Linear regression analysis was used for estimating the relationship between optical density at 600 nm (OD_{600}) and CFU/ml. Prediction was made within the range of values in the dataset used for model-fitting.

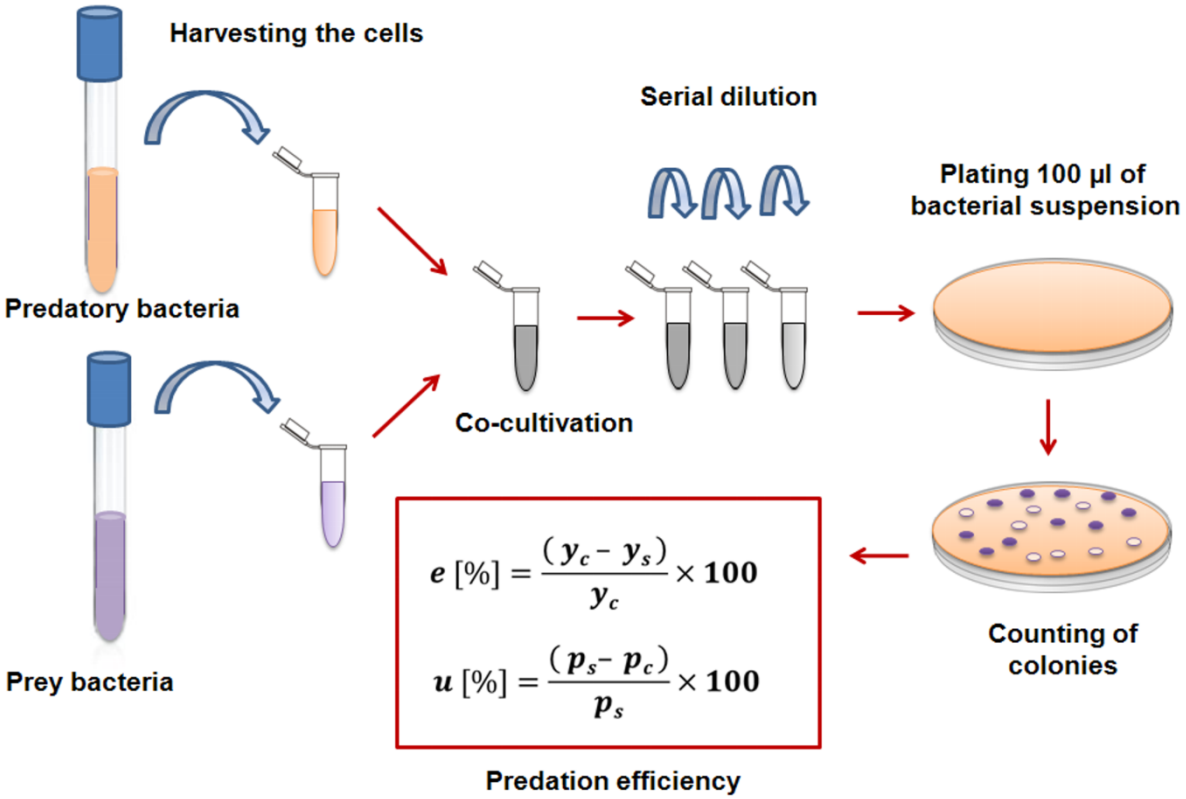

Figure S4. Work flow for the CFU-based predation assay and equations to calculate the predator's killing efficiency (*e*) and its utilization of prey (*u)*. The variables were defined as follows: y_c = colony-forming units of the prey bacterium that had been grown in the absence of a predator; y_s = colony-forming units (CFUs) of the prey bacterium that had been cocultured with a predator; p_c = CFUs of the predatory bacterium that had been grown in the absence of prey; p_s = CFUs of the predatory bacterium that had been cocultured with a prey bacterium.

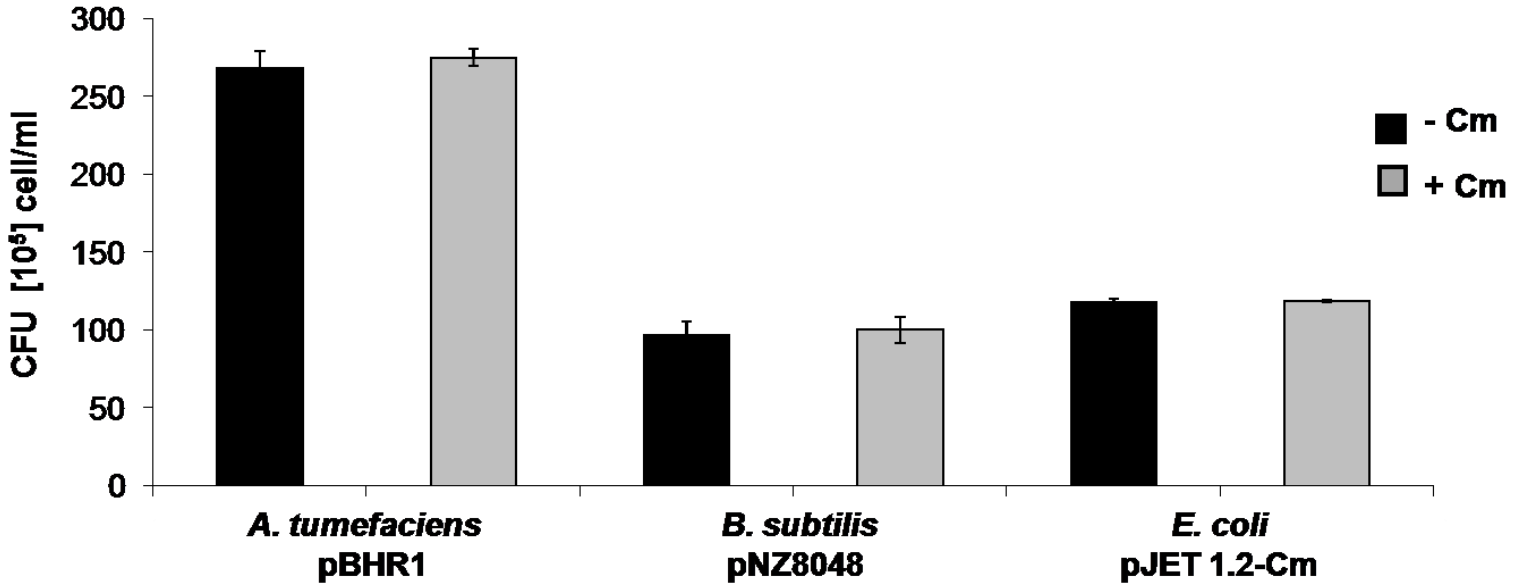

Figure S5. Plasmid stability analysis of *A. tumefaciens/*pBHR1, *B. subtilis/*pNZ8048, and *E. coli/*pJET1.2-cf. Bacteria were cultured in 5 ml LB medium supplemented with chloramphenicol (25 µg ml^{-1}). After 48 h, 2 ml of each bacterial culture were harvested by centrifugation (1,200 g, 4 °C, 5 min). The supernatant was removed and the cell pellet was washed three times with 2 ml of PBS buffer and, finally, resuspended in 1.6 ml of PBS buffer. From these suspensions, 370 µl aliquots (cell concentration adjusted to 1 x 10^6 cells ml^{-1}) were mixed with the same amount of either PBS buffer or PBS buffer supplemented with chloramphenicol (25 µg ml^{-1}). Incubation was then continued for 24 h at 30 °C, before serial dilutions of the cultures were prepared and spread on LB agar plates containing chloramphenicol (25 µg ml^{-1}). The CFU number was determined, as described for the CFU-based predation assay. None of the tested strains showed a significant plasmid loss, which is consistent with previous studies (cf. **Weber AE, San K-Y.** 1990. Population dynamics of a recombinant culture in a chemostat under prolonged cultivation. Biotechnol. Bioengineering. **36**:727-736).

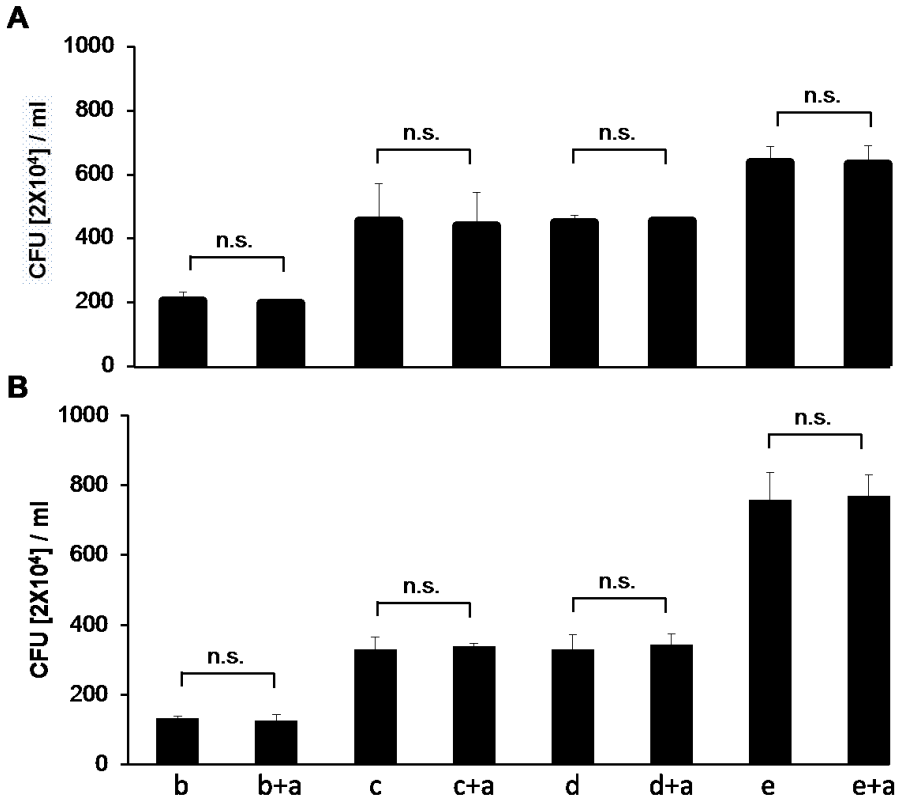

Figure S6. Testing of *Myxococcus fulvus* in the CFU-based predation assay. Mean (±95% confidence interval) CFU of (b) *B. subtilis*, (c) *C. pseudoviolaceum*, (d) *E. coli* and (e) *R. rhodochrous* grown in the absence or presence of (a) the predatory bacterium *M. fulvus*. Monocultures of prey served as controls to assess the reduction efficiency after (A) 24 h, and (B) and 48 h (n.s., not significant).

Table S1. Swarm expansion in the lawn predation assay. Mean (± 95% confidence interval, n=3) of the swarm diameter [in mm] on each prey bacterium after one and ten days (d) of incubation.

	Prey species									
	Bacillus subtilis		*C. pseudoviolaceum*		*Escherichia coli*		*Micrococcus luteus*		*R. rhodochrous*	
Predator species	**1 d**	**10 d**	**1 d**	**10 d**	**1 d**	**10 d**	**1 d**	**10 d**	**1 d**	**10 d**
Myxococcus fulvus	4.0 ± 1.0	14.6 ± 0.6	5.0 ± 1.2	6.0 ± 1.3	8.0 ± 1.0	42.6 ± 2.8	9.0 ± 1.0	24.6 ± 5.9	6.0 ± 1.0	8.0 ± 1.0
Lysobacter capsici	8.3 ± 0.6	9.6 ± 0.7	7.0 ± 1.0	7.4 ± 1.0	6.0 ± 1.0	7.6 ± 0.6	9.6 ± 1.3	10.3 ± 0.6	7.4 ± 1.0	7.4 ± 1.0
Lysobacter enzymogenes	7.0 ± 0.2	7.6 ± 0.7	4.6 ± 0.6	5.6 ± 0.6	9.6 ± 1.3	24.3 ± 1.3	8.0 ± 1.0	11.3 ± 0.6	7.4 ± 1.0	12.3 ± 0.6
Lysobacter oryzae	9.0 ± 0.2	9.6 ± 0.6	7.0 ± 1.0	7.0 ± 1.0	9.3 ± 0.6	9.6 ± 0.7	8.6 ± 1.7	9.6 ± 0.6	8.0 ± 1.0	8.0 ± 1.0

Table S2. Evaluation of predation efficiency in the CFU-based predation assay (*e* = killing efficiency; *u* = prey utilization; n.d. = not determined).

	Prey species							
	Agrobacterium tumefaciens		*Bacillus subtilis*		*C. pseudoviolaceum*		*Escherichia coli*	
Predator species	**e [%]**	***u* [%]**	**e [%]**	***u* [%]**	**e [%]**	***u* [%]**	**e [%]**	***u* [%]**
Lysobacter capsici	14.0 ± 1.0	n.d.	96.5 ± 0.4	19.0 ± 1.5	90.0 ± 1.5	23.7 ± 0.2	19.0 ± 1.5	3.8 ± 1.2
Lysobacter enzymogenes	8.5 ± 0.1	n.d.	62.6 ± 1.8	1.0 ± 0.5	9.4 ± 0.1	9.6 ± 0.4	14.6 ± 0.7	8.3 ± 1.5
Lysobacter oryzae	12.0 ± 0.2	n.d.	98.1 ± 1.0	15.3 ± 1.5	100.0 ± 0.0	26.6 ± 0.1	20.0 ± 1.8	13.4 ± 2.5

	Prey species							
	Lactococcus lactis		*Pseudomonas fluorescens*		*Ralstonia solanacearum*		*Rhodococcus rhodochrous*	
Predator species	**e [%]**	***u* [%]**	**e [%]**	***u* [%]**	**e [%]**	***u* [%]**	**e [%]**	***u* [%]**
Lysobacter capsici	68.4 ± 7.5	16.2 ± 1.5	1.0 ± 0.1	n.d.	2.69 ± 2.0	n.d.	97.6 ± 0.9	21.6 ± 0.4
Lysobacter enzymogenes	99.0 ± 1.5	13.7 ± 0.36	1.2 ± 0.1	n.d.	0.5 ± 1.0	n.d.	4.2 ± 0.4	10.6 ± 2.2
Lysobacter oryzae	99.1 ± 1.5	17.7 ± 0.7	1.4 ± 0.6	n.d.	11.6 ± 7.6	n.d.	96.8 ± 0.8	20.0 ± 0.1