SUPPLEMENTARY INFORMATION FOR

Engineering a predatory bacterium as a proficient killer agent for intracellular bio-products recovery: The case of the polyhydroxyalkanoates

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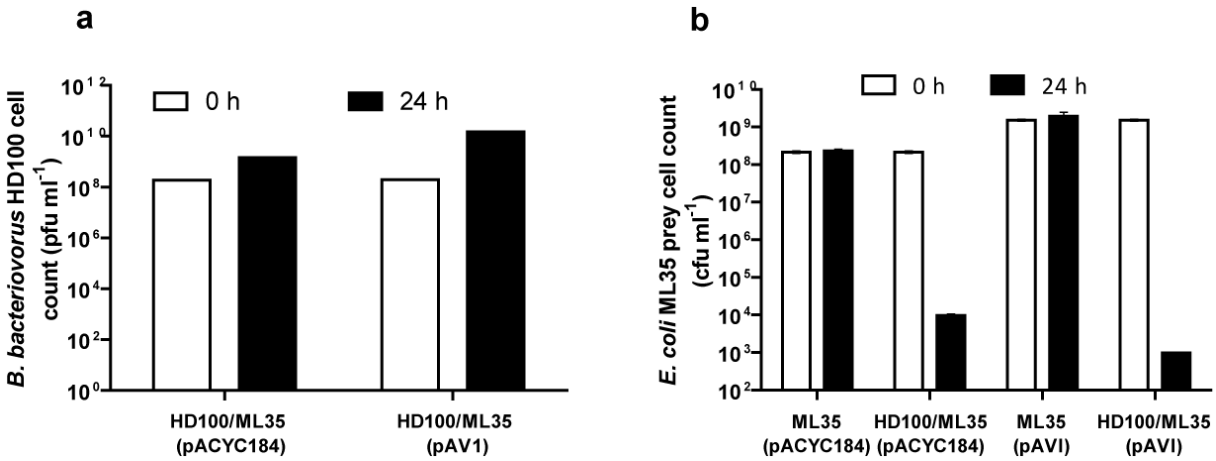
Supplementary Table 1. Bacterial strains, plasmids and oligonucleotides used in this study.

Strain, plasmid or oligonucleotide	Genotype, description or sequence (5' to 3')	Reference
Strain		
B. bacteriovorus		
HD100	Type strain, genome sequenced	1,2
Bd3709	*Bd3709*::pK18*mob* mutant, HD100 derivative strain	This work
Bd2637	Δ*Bd2637* mutant, HD100 derivative strain	This work
***C. necator* H16**	Wild-type	3
E. coli		
ML35	*E. coli* B, *lacYI-*	Jurkevitch lab collection
S17-λ*pir*	*Tra*$^{+}$ *recA pro thi hsdR chr::RP4-2*	4
P. putida		
KT2440	*P. putida* mt-2 without TOL plasmid, *hsdR*	5
KT42Z	phaZ disruptional mutant, Km^{r}, KT2442 derivative strain	6
Plasmid		
pACYC184	p15A, Cm^{R}, Tet^{R}	8
pAV1	pACYC184 derivative containing PHB biosynthetic genes (*phbABC*) from *C. necator* H16, Cm^{R}	Prieto lab collection
pK18*mob*sacB	Km^{R}, ColE *ori*V, Mob+, *lac*Za, *sacB*; vector for allelic exchange homologous recombination mutagenesis	9
pK18*mob*sacB-2637	pK18*mob*sacB derivative used for Bd2637 deletion	This work
pK18*mob*	*ori*ColE1 Mob^{+} *lacZα*$^{+}$, Km^{R} used for direct insertional disruption	9
pK18*mob*-3709	pK18*mob* derivative used for Bd3709 disruption	This work
Oligonucleotide[a]		
PHB-F	GCTCTAGAGACGTCCCAACCACCTCTTGAA	This work
PHB-IR	CGGGATCCGGTGACCTCCTGATGAAGAAATCA	This work
PHB-IF	CGGGATCCTGATTACGTCAGCAAATCCGG	This work
PHB-R	CCCAAGCTTGTTGTGAAACAGATTGCGCCC	This work
PHO-F	GCTCTAGAG GAGCTTCTTCCAGCCATTCGTGA CGAGC	This work
PHO-R	CCCAAGCTTCGGGATGACCCACGGTCATTC	This work

[a] Engineered endonuclease sites in the oligonucleotides are shown underlined.

Supplementary Table 2. Molecular characterization of mcl-PHA and PHB polymers.

Strain	Mn (kDa)	Mw (kDa)	PDI
KT2440 control	53.37 ± 7.54	100.37 ± 6.81	1.89 ± 0.13
HD100/KT2440	50.71 ± 4.48	96.41 ± 16.30	1.89 ± 0.16
Bd3709/KT2440	76.56 ± 11.30	124.80 ± 25.48	1.62 ± 0.15
HD100/KT42Z	82.57 ± 10.56	42.78 ± 10.32	1.73 ± 0.14
Bd3709/KT42Z	57.98 ± 6.59	88.57 ± 9.56	1.53 ± 0.13
ML35 (pAV1) control	2000 ± 300	3000 ± 200	1.8 ± 0.3
HD100/ML35 (pAV1)	600 ± 40	1060 ± 5	1.8 ± 0.1
Bd2637/ML35 (pAV1)	1000 ± 100	2000 ± 300	1.7 ± 0.1



Supplementary Figure 1. Influence of prey PHB content on fitness of *B. bacteriovorus* HD100. (**a**) Number of viable cells of *B. bacteriovorus* HD100 at the start of the experiment (time zero) and after 24 h preying upon *E. coli* ML35 cells accumulating PHB [ML35 (pAV1)] or devoid of it [ML35 (pACYC184)], previously adjusted to 0.3 g l^{-1} of residual biomass. (**b**) Number of viable cells of *E. coli* ML35 accumulating PHB [ML35 (pAV1)] or devoid of it [ML35 (pACYC184)] at the start of the experiment (time zero) and after 24 h of predation with *B. bacteriovorus* HD100.

Supplementary movies:

Movie 1. *B. bacteriovorus* Bd3709 mutant preying on PHA-accumulating *P. putida* KT2440 at the onset of predation (time zero).

Movie 2. *B. bacteriovorus* Bd3709 mutant preying on PHA-accumulating *P. putida* KT2440 after 24 h of predation.

Supplementary references

1. Stolp, H. & Starr, M. P. *Bdellovibrio bacteriovorus* Gen. et Sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. *Antonie Van Leeuwenhoek* **29,** 217–248 (1963).
2. Rendulic, S. *et al*. A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. *Science* **303,** 689–692 (2004).
3. Poehlein, A., Kusian, B., Friedrich, B., Daniel, R. & Bowien, B. Complete genome sequence of the type strain *Cupriavidus necator* N-1. *J. Bacteriol.* **193,** 5017 (2011).
4. Simon, R., Priefer, U. & Pühler, A. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram-negative bacteria. *Nat. Biotechnol.* **1,** 784–791 (1983).
5. Bagdasarian, M. *et al*. Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors for gene cloning in *Pseudomonas*. *Gene* **16,** 237–247 (1981).
6. de Eugenio, L.I. *et al*. Biochemical evidence that *phaZ* gene encodes a specific intracellular medium chain length polyhydroxyalkanoate depolymerase in *Pseudomonas putida* KT2442: characterization of a paradigmatic enzyme. *J. Biol. Chem.* **282,** 4951– 4962 (2007).
7. Chang, A. C. Y., & Cohen, S. N. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *J. Bacteriol.* **134,** 1141–1156 (1978).
8. Schäfer, A. *et al*. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. *Gene* **145,** 69–73 (1994).