Predation Efficiency upon Clinical Isolates: *Bdellovibrio bacteriovorus* is Prey Specific and Origin Dependent

Claudia Saralegui[1†], Cristina Herencias[2*†], Ana Halperin[1], Juan de Dios-Caballero[1], Blanca Pérez-Viso[1], Sergio Salgado-Briegas[3], Val F. Lanza[1], Rafael Cantón[1], Fernando Baquero[1], María Auxiliadora Prieto[3], Rosa del Campo[1*],

[1]Department of Microbiology, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigacion Sanitaria (IRYCIS), Madrid, Spain.

[2]Microbial Biotechnology Department, Centro Nacional de Biotecnología, CSIC, Madrid, Spain, C/ Darwin 3, 28049, Madrid Spain

[3]Microbial and Plant Biotechnology Department, Biological Research Center-Margarita Salas, CSIC, Ramiro de Maeztu 9, 28040 Madrid, Spain.

[†] These authors contributed equally to this work and should be considered both as first authors.

[*]Corresponding author: Cristina Herencias and Rosa del Campo

Mailing address: cherodr@gmail.com (Cristina Herencias) and rosacampo@yahoo.com (Rosa del Campo).

ABSTRACT

The use of predatory bacteria as live antibiotics has been proposed for managing bacterial infections, especially for those caused by antibiotic multiresistant isolates for which there are few therapeutic options. However, the current knowledge in this field is scarce, with most of the available data based on environmental isolates, with a significant lack of human clinical samples. In this study, we evaluated the predatory spectrum of the reference strain *Bdellovibrio bacteriovorus* 109J on 13 *Serratia marcescens* (5 of which were carbapenemase producers) and 78 *Pseudomonas aeruginosa* clinical isolates from respiratory (colonizing the lungs of patients with cystic fibrosis) or bacteremic infections, differentiated by phenotype (mucoid or not), antibiotic resistance phenotype (including multidrug-resistant isolates), and genetic lineage (frequent and rare sequence types). The source of the isolates was significantly associated with predation efficiency (100% for *S. marcescens*, 67% for *P. aeruginosa* from cystic fibrosis, and 25% for *P. aeruginosa* from bacteremia). In contrast, no correlation with colonial morphotype, genetic background, or antibiotic susceptibility was found. To evaluate the influence of the predator on the predation event, we employed a more aggressive *B. bacteriovorus* mutant 109J preying upon the same 48 bacteremic *P. aeruginosa* isolates. The mutant's predation efficiency was higher than that of their wild-type counterpart (43% vs. 25%), pointing out that predation is specific to each prey-predator pair of isolates. Our results provide the most extensive study of clinical prey susceptibility published to date and show that the prey-predator interaction is influenced by the origin of the isolates rather than by their genetic background or their antibiotic susceptibility phenotype.

IMPORTANCE

The potential usefulness of predatory bacteria in controlling human pathogens, particularly those that are multiresistant to antibiotics, is enormous. Although this possibility has long been suggested, there are still no data on predation susceptibility in clinical strains, and the possible presence of autochthonous predators of the human microbiota has not been investigated. In this study, we employed a reference predator with an environmental origin to study predation phenomena in 3 well-characterized collections of human clinical isolates. Our results demonstrated that predation is a specific consequence of each prey-predator interaction, with the origin of the strains the most relevant factor. In contrast, the genetic background, morphotype, and antibiotic resistance did not appear to influence the predation phenomenon. We also highlight the involvement of a putative polyhydroxyalkanoate depolymerase protein of *B. bacteriovorus* in determining prey susceptibility. To our knowledge, this study is the largest performed with strains of clinical origin, discriminating between various genera and including strains with multiresistance to antibiotics.

INTRODUCTION

The “golden age of antibiotics” in the mid-20th century was followed by the emergence of pathogens resistant to almost all available antibiotics, leading to the current global crisis of multidrug-resistant (MDR) bacteria (1, 2). To identify successful alternative antimicrobial therapies, bacterial pathogenicity needs to be understood as a multifactorial issue in which the surrounded microbiota, which includes natural competitors and predators, is also involved (3). In nature, predatory bacteria play an important role in maintaining population sizes by linking the production and removal of biomass in microbial communities, which in turn promotes the diversity of microorganisms and contributes to the global stabilization of the

ecosystem (4, 5). The ecological role of predators could also be refined and exploited in the fight against clinical pathogens, given that the predators represent dynamic microorganisms that experience (as do their opponents) continuous physical, morphological, and metabolic adaptations, altering their behavior to counteract each other. This evolutionary reciprocity is the basis of coevolution, where adaptation by one player not only promotes change in its opponent, but the opponent's adaptation likewise generates selection as an evolutionary response to the first player (6). Increasing our understanding of how the microbiota community ecology is balanced will contribute to the selection of biocontrol agents that target pathogenic bacteria for which antibiotics are not an alternative due to multiresistance (7–9). The successful development of predatory bacteria as “living antimicrobial agents” and a complete understanding of the predation mechanism depend on the characterization of their phenotypical predation preferences, mainly their prey range. A predator might have a wide repertoire of susceptible prey, but predation appears to be strongly strain-specific, fundamentally based on the composition of the prey cell envelope, and influenced by environmental conditions (10–12). However, addressing the specific factor driving prey preference and susceptibility is challenging and has so far remained elusive, particularly in non-environmental bacterial collections (13, 14).

The most studied bacterial predators are *Bdellovibrio* and like organisms (BALOs), which are small vibrioids, rod-shaped gram-negative aerobic bacteria, recently reclassified to the class of *Oligoflexia*, which belongs to the *Proteobacteria* phylum (15). Its known prey species spectrum includes genera of *Proteobacteria* phylum as *Pseudomonas*, *Escherichia*, *Acinetobacter*, *Aeromonas*, *Burkholderia*, *Citrobacter*, *Enterobacter*, and *Klebsiella* (11, 16–18), as well as antibiotic-resistant isolates (19). Although BALOs were first isolated from soil,

89 they are ubiquitous in nature and can be found in aquatic and terrestrial environments,
90 including hypersaline systems (20), biofilms (21), mammalian intestines (22–24), and the lungs
91 of patients with cystic fibrosis (25). In addition to the genetic detection of BALOs, several
92 authors have documented the *in vivo* phenomenon of predation in human microbial
93 ecosystems (26–29).
94 Herein, we characterized the predation susceptibility and efficiency of the reference strain
95 *B. bacteriovorus* 109J against human clinical *Serratia marcescens* and *Pseudomonas*
96 *aeruginosa* isolates, of diverse origins, genetic backgrounds, and antibiotic-resistant
97 phenotypes. We also explored the relevance of the predator role on predation using a more
98 aggressive mutant, a previously described derivative of *B. bacteriovorus* 109J (30). We found
99 a specific recognition of susceptible prey that could be related to its single deletion genotype
100 in the bd2637 gene, coding for a putative polyhydroxyalkanoate (PHA) depolymerase enzyme.
101
102 **RESULTS**
103 **Prey origin determines the predation susceptibility to *B. bacteriovorus* 109J.** The use of
104 predatory bacteria as biocontrol agents depends on their efficiency in eradicating bacterial
105 populations and on which bacterial species are susceptible to predation, also known as the
106 prey range. We measured the predation susceptibility of *S. marcescens*, CF-PA and BACT-PA
107 clinical isolates by monitoring the decrease in OD_{600} of the predation co-cultures and by
108 measuring the viable prey cell number.
109 All 13 *S. marcescens* isolates tested were preyed on by *B. bacteriovorus* 109J, whereas CF-PA
110 isolates were significantly more susceptible to predation (20 out of 30, 67%) than BACT-PA (12
111 out of 48, 25%) (p<0.02) (Fig 1, S1, S2, and S3). No correlation between predation and

P. aeruginosa genetic lineage was observed, with discrepancies in 6 of the 12 STs grouping more than one isolate [ST175 (1/3), ST253 (1/3), ST274 (1/2), ST532 (1/2), ST646 (2/3), and ST1017 (1/2)] (Table S1). There was also no correlation with the mucoid (4/7) or non-mucoid phenotype of CF isolates (16/23) (Fig S4) or with the antibiotic susceptibility phenotype (Fig 2 and Table S2).

There were significant differences regarding quantitative predation (as measured by the differences in median PR) among the predation-susceptible isolates from each collection (Table S1). The median PR was 2.22 and 3.91 for *S. marcescens* and CF-PA (Mann-Whitney test $p = 0.02$), respectively, and 1.34 for BACT-PA (Mann-Whitney test $p < 0.03$). The analysis of the predation kinetics of the curves, MKR and area under the curve (AUC) did not correlate significantly with the predation rate of each isolate (Fig S5, Table S1, and Table S3).

Influence of the predator's predation capacity. The prey or predator determinants responsible for predation specificity have not yet been elucidated. However, the hydrolytic arsenal of *B. bacteriovorus* plays a crucial role in predation efficiency, given that it determines the success of their lifecycle (30, 41). Characterization of each predator's specific prey spectrum is a requirement for the clinical use of predators as living antimicrobial agent. Studies have reported that different BALO lineages and predators isolated from different niches have different prey spectra (42, 43).

Interestingly, a more aggressive *B. bacteriovorus* 109J derivative has been designed and it increased by threefold the killing efficiency of the wild type preying upon *E. coli* bacterial populations (30). This strain was constructed by a single deletion of the gene bd2637 (coding for a PHA depolymerase) and WGS revealed that no significant chromosomal mutations were accumulated during the gene deletion process (Table S4).

We compared the predation capacity and specificity between the wild type *Bdellovibrio* and this single-gene mutant strain among the 48 BACT-PA isolates, which were the less susceptible prey collection. As expected from previous studies (30), the mutant Bd2637 strain had a higher predation frequency than the wild strain (21/48, 43.8% vs. 12/48, 25.0%); however, the differences between the PR values did not reach statistical significance (1.8 vs. 1.3, Mann-Whitney test $p = 0.6$) (Table S1, Fig 1D and S6). Interestingly, the repertoire of susceptible prey was completely different: 16 out of the 48 (33.3%) BACT-PA isolates were resistant to both predators, 15 (31.2%) were susceptible only to the mutant, 7 (14.6%) were susceptible only to the wild-type strain, and 6 (12.5%) were preyed on by both predators (Fig 3). The observed discrepancies were consistently observed in the replicates of each experiment and were not associated with any particular prey characteristic. Among the common susceptible preys (e.g., BACT1, BACT46, and BACT195), the effectiveness of each predator was strain specific. Again, the antibiotic resistance profile and the analysis of the kinetics parameters of the predation curves showed no correlation with predation susceptibility or PR (Fig 2D and S5). Thus, only the deletion of the catalytic activity derived from the bd2637 gene and the associated effects were responsible for the changes in prey susceptibility.

DISCUSSION

The use of BALOs as biological control agents in environmental and medical microbiological settings (22, 44) has been suggested based on their lack of interaction with human cells (45). As occurs with antibiotics, testing the individual *in vitro* susceptibility for prey and predator pairs of strains is a requirement, mainly when predation could be substantially affected by environmental or biological conditions, as we postulate herein. Predators have been studied primarily within a free environmental context, given that the knowledge on predation

158 susceptibility of clinical isolates is much more limited. Although human pathogens are highly
159 diverse, we focused our research on *Pseudomonas* and *Serratia* genera due to their ubiquity
160 in nature, their high frequency in human diseases being also carrying antibiotic resistance
161 genes, and the availability of previously well-characterized collections including both frequent
162 and infrequent lineages. However, the qualitative discrepancies between isolates grouped in
163 the same ST but from different sources, indicated that the previous adaptation to these
164 environments influences the predation process.
165 A single bacterial species (or ST) can be found in different habitats, as environmental and
166 nosocomial niches (human microbiota of patients, built environments). Nevertheless,
167 population genetic studies have revealed differing genetic evolutionary processes, in
168 particular for those lineages highly adapted to hospital conditions, which are also known as
169 high-risk clones, as is the case for *P. aeruginosa*, which colonizes the airways of CF-patients
170 and are close to strains of environmental origin, as we have previously shown (46). A notable
171 result of our study is the higher susceptibility of CF isolates to predation, without correlation
172 with the morphological growth (mucoid vs. non-mucoid), genotype (absence of correlation
173 with STs), or antibiotic susceptibility patterns. The presence of highly specialized predators in
174 human microbiota cannot be ruled out, given that all available data have focused on
175 environmental predators. Our microbial ecosystems, however, probably have the same rules
176 of population control based on predation. Predators with a human origin could be more
177 suitable for limiting well-adapted human pathogens (25).
178 In a complex and diverse ecosystem, preference for particular prey would be a dynamic
179 feature (47). Although experiments are often conducted using individual lineages, the use of
180 mixed populations should be a future goal to validate prey specificity in a community and the

consequences on microbial population structure. This work indicates that quantitative predation, determined as relative predation rates (PR) between members of a community, could be critical to understand the dynamics of bacterial ensembles composed by different preys and predators. All *S. marcescens* isolates were susceptible to predation, as previously reported (27), and the PR was significantly higher compared with *P. aeruginosa* isolates. This finding is in line with a previous report showing a limited ability of some *B. bacteriovorus* to prey on *P. aeruginosa* (48).

The contribution of the predators' genetic background is a pending issue. Thus far, only 8 *B. bacteriovorus* genomes have been entered into public databases, all of them from an environmental origin. To elucidate that, we used the mutant Bd2637 that was found to be a more aggressive phenotype than the wild-type *B. bacteriovorus* 109J (30). The genotype of the Bd2637 strain corresponds to a single deletion of the gen bd2637, which encodes a putative PHA depolymerase responsible for the degradation of biopolymers. The analysis of the amino acid sequence of this enzyme revealed that, apart from the characteristic esterase catalytic domain type 2 (49), the N-terminal sequence possesses a peptidase-like domain (50). This structure would provide a Bd2637 enzyme, which might act as a promiscuous enzyme with the proper catalytic architecture to act on extracellular specific components of the prey (outer membrane components, extracellular matrix, or capsid), thereby promoting specific predation. This specificity could explain why the predator is unable to complete or even begin the predatory cycle and could help identify predation resistance factors. Thus, the selection of the appropriate predator for specific prey, which needs a larger and more in-depth study on predation, would be overcome with the rational use of broad prey spectrum predators. It is worth noting that the prey range does not depend only on the prey susceptibility but also

on the predator specificity, which highlights the importance of predator-prey interaction and co-evolution to overcome predation and resistance, respectively (51).

In summary, we conclude that the phenomenon of predation is defined by the particularities of both prey and predator isolates and is conditioned by environmental factors. There is a possible source dependence, and the presumption of predation cannot be inferred from different isolates of the same species, even within the same genetic lineage. To define an ecological alternative to antibiotics, the possible existence of predators within the human microbiota should be explored.

MATERIALS AND METHODS

Strains and growth conditions. The *B. bacteriovorus* 109J reference strain and its PHA-depolymerase mutant *B. bacteriovorus* 109J-bd2637 were used as predators in our experimental system (30). Prey (n=91) were selected from previously well-characterized collections of *S. marcescens* colonizing or infecting patients (neonates and adults) admitted to intensive care units (n=13) (31, 32). *P. aeruginosa* isolated from the airways of patients with cystic fibrosis (hereafter CF-PA, n=30) (33), and invasive isolates causing bacteremic infections (hereafter BACT-PA, n=48) (34). The inclusion criterion for the *S. marcescens* isolates, 5 of which were carbapenemase producers, was the availability of their whole-genome sequence. CF-PA and BACT-PA isolates were selected based on their genetic background discriminated by sequence type (ST), including both frequent and rare STs, as well as by their antibiotic susceptibility, including MDR isolates. Colony morphology was only considered in CF-PA isolates as mucoid (n=7) and non-mucoid isolates (n=23). Relevant data on all isolates is shown in Tables S1 and S2.

Predators were routinely grown (as described previously) by co-cultivation with *Pseudomonas putida* KT2440 in Difco nutrient broth (DNB) (0.8 g/l nutrient broth at pH 7.4) or HEPES buffer (25 mM at pH 7.8) supplemented with 2 mM $CaCl_2 \cdot 2H_2O$ and 3 mM $MgCl_2 \cdot 3H_2O$ and were further purified by filtering twice through a 0.45-µm filter. The prey strains were cultivated on Luria Broth (LB) for 16 h at 37 ºC and were further diluted in HEPES buffer to an optical density at 600 nm of 1 (OD_{600} 1) for the subsequent experiments.

Genetic background of prey and predators. Whole-genome sequencing (WGS) of both predator strains was performed on the Illumina HiSeq 4000 platform following the specification of Microbes NG (https://microbesng.com/). WGS data is available at BioProject ID: PRJNA723206 (https://www.ncbi.nlm.nih.gov/sra/PRJNA723206) and Genbank accession numbers: SRX10641169 and SRX10641170 for *B. bacteriovorus* 109J and the Bd2637 mutant, respectively. Genome comparisons were performed using *minimap2* (35)and *paftools* to align and variant calling respectively. Variant calling parameters were set to 500 bp minimum length to compute coverage and variant. Mutations were manually inspected with Artemis (36).

A tree showing the genetic relationship of 457 genomes of *S. marcescens* was constructed by combining a previously published tree (31) and 5 additional genomes of the carbapenemase-producing isolates (32). The analysis was performed by the cano-wgMLST_BacCompare web-based tool (37), and the final tree was edited using the iTOL v4.4.2 web-based tool (38). STs of the *P. aeruginosa* isolates were depicted as a minimum-spanning tree by the PHYLOViZ tool using the 7 concatenated sequences of all isolates available in May 2021 in https://pubmlst.org/organisms/pseudomonas-aeruginosa.

Predation experiments. The predation experiments included measuring predator and prey viability and monitoring cell density (OD_{600}) for 24 h. Predation co-cultures were prepared in

HEPES buffer and inoculated with a *Bdellovibrio* inoculum of 10^9 plaque-forming units/ml (PFU/ml) and a prey inoculum adjusted to an OD_{600} of 0.3. Predator and prey strain viabilities were calculated from the co-culture containing both strains. *B. bacteriovorus* strain viabilities (measured in PFU/ml) were calculated by performing serial dilutions from 10^{-1} to 10^{-7} in HEPES buffer and developing on the lawn of prey bacteria after 48–72 h of incubation at 30 °C using the double-layer method (39, 40). Briefly, 0.1 ml of the appropriate dilution was mixed with an additional 0.5 ml prey cell suspension of *P. putida* KT2440 pre-grown in LB and prepared in HEPES Buffer at pH 7.8 at OD_{600} 10, vortexed and plated on DNB solid medium. To calculate prey viable cell counts (clinical isolates), 10 µl of each dilution was plated on LB solid medium and colony-forming units (CFU/ml) were counted after 24 h of incubation at 37 ºC.

Prey-predator co-cultures were performed on 96-microwell plates at a final volume of 200 µl and incubated for 24 h at 30 ºC with orbital shaking in a Synergy HTX (BioTek). Two conditions were tested for each prey: the growth control well without the predator and the predation well with the mixture of prey and predator. The prey's dynamic survival was monitored by measuring OD_{600} every 10 min, counting the viable cells at the end of the experiment by seeding on LB agar plates. Each prey was tested in at least 2–3 independent biological replicates, and the results corresponded to the mean values of all experiments.

The predation rate (PR) was calculated as the ratio of viable cells at the control well to the predation well at the end of the experiment expressed in log10 values. Positive predation is considered when this rate was >0.5. The predation kinetics encompassed the area under the curve (AUC) and the maximum killing rate (MKR). The AUC parameter was obtained using the 'auc' function from the 'flux' R package. The MKR value corresponds to the slope of the predation curve: the more negative the MKR, the more efficient the predation. The MKR was

calculated as the opposite value of µmax (maximum growth rate in typical growth curves), which was obtained from the 'growthrates' R package. Data are represented using an R custom script and the 'ggplot2' package.

UMAP clustering. UMAP was used to visualize the clustering distribution of the antibiotic resistance profile and predation susceptibility. The statistical analysis was performed in R version 3.5.0, and plotting was performed using ggplot version 2.2.1.

Statistical analysis. Data sets were analyzed using Prism 6 software (GraphPad Software Inc.) and RStudio software v.1.2.5001. An unpaired t-test followed by Welch's correction was performed to compare the prey's PR. A non-parametric paired Wilcoxon test was performed to compare the MKR values in control versus predation wells, and the Kruskal-Wallis test was performed to compare PR values between collections, after assuming non-normality with the Shapiro-Wilk normality test. Lastly, the differences in predation frequency between the CF and BACT-PA collections were explored using the chi-squared test.

ACKNOWLEDGMENTS

We appreciate the technical support of Natalia Huertas. CH is supported by Comunidad Autónoma de Madrid (PEJD-2018- POST/BMD-8016). CS is granted by "Fundación Mutua Madrileña" achieved in 2017 call by RDC (AP165902017). SSB is a recipient of a predoctoral FPU grant (FPU17/03978) from the Spanish Ministry of Universities. This work was supported by the Instituto de Salud Carlos III, PI17/00115 and PI20/00164 to RdC, REIPI (RD16/0016/0011) actions, co-financed by the European Development Regional Fund "A way to achieve Europe" (ERDF), and Vertex Pharmaceuticals.

296 **COMPETING INTERESTS**

297 RdC was the recipient of a Vertex Pharmaceuticals grant. The other authors declare no conflict
298 of interest.

299

300 **REFERENCES**

301 1. Cavallo FM, Jordana L, Friedrich AW, Glasner C, van Dijl JM. 2021. *Bdellovibrio*
302 *bacteriovorus*: a potential 'living antibiotic' to control bacterial pathogens. Crit Rev
303 Microbiol 0:1–17.
304 2. Baquero F. 2021. Threats of antibiotic resistance: an obliged reappraisal. Int Microbiol
305 1.
306 3. Celis AI, Relman DA. 2020. Competitors versus Collaborators: Micronutrient processing
307 by pathogenic and commensal human-associated gut bacteria. Mol Cell 78:570–576.
308 4. Fuhrman JA, Caron DA. 2016. Heterotrophic planktonic microbes: virus, bacteria,
309 archaea, and protozoa https://doi.org/10.1128/9781555818821.ch4.2.2.
310 5. Thompson JN. 1999. The evolution of species interactions. Sci (washingt D C) 284:2116–
311 2118.
312 6. Gallet R, Tully T, Evans MEK. 2009. Ecological conditions affect evolutionary trajectory
313 in a predator-prey system. Evolution (N Y) 63:641–651.
314 7. Madhav M, Baker D, Morgan JA., Asgari S, James P. 2020. Wolbachia: A tool for livestock
315 ectoparasite control. Vet Parasitol 288:109297.
316 8. Legein M, Smets W, Vandenheuvel D, Eilers T, Muyshondt B, Prinsen E, Samson R,
317 Lebeer S. 2020. Modes of action of microbial biocontrol in the phyllosphere. Front
318 Microbiol 11.

9. Shen Y, Loessner MJ. 2021. Beyond antibacterials – exploring bacteriophages as antivirulence agents. Curr Opin Biotechnol 68:166–173.

10. Lambert C, Lerner TR, Bui NK, Somers H, Aizawa S-I, Liddell S, Clark A, Vollmer W, Lovering AL, Sockett RE. 2016. Interrupting peptidoglycan deacetylation during Bdellovibrio predator-prey interaction prevents ultimate destruction of prey wall, liberating bacterial-ghosts. Sci Rep 6:26010.

11. Jurkevitch E, Davidov Y. 2006. Phylogenetic diversity and evolution of predatory prokaryotes ACS Division of Fuel Chemistry, Preprints.

12. Pernthaler J. 2005. Predation on prokaryotes in the water column and its ecological implications. Nat Rev Microbiol 3:537–546.

13. Li N, Wang K, Williams HN, Sun J, Ding C, Leng X, Dong K. 2017. Analysis of gene gain and loss in the evolution of predatory bacteria. Gene 598:63–70.

14. Duncan MC, Gillette RK, Maglasang MA, Corn EA, Tai AK, Lazinski DW, Shanks RMQ, Kadouri DE, Camilli A. 2019. High-Throughput analysis of gene function in the bacterial predator *Bdellovibrio bacteriovorus*. MBio 10.

15. Waite DW, Chuvochina M, Pelikan C, Parks DH, Yilmaz P, Wagner M, Loy A, Naganuma T, Nakai R, Whitman WB, Hahn MW, Kuever J, Hugenholtz P. 2020. Proposal to reclassify the proteobacterial classes Deltaproteobacteria and Oligoflexia, and the phylum Thermodesulfobacteria into four phyla reflecting major functional capabilities. Int J Syst Evol Microbiol 70:5972–6016.

16. Chanyi RM, Ward C, Pechey A, Koval SF. 2013. To invade or not to invade: two approaches to a prokaryotic predatory life cycle. NCR Res Press 59:273–279.

17. Russo R, Chae R, Mukherjee S, Singleton E, Occi J, Kadouri D, Connell N. 2015.

342 Susceptibility of select agents to predation by predatory bacteria. Microorganisms
343 3:903–912.
344 18. Baker M, Negus D, Raghunathan D, Radford P, Moore C, Clark G, Diggle M, Tyson J,
345 Twycross J, Sockett RE. 2017. Measuring and modelling the response of *Klebsiella*
346 *pneumoniae* KPC prey to *Bdellovibrio bacteriovorus* predation, in human serum and
347 defined buffer. Sci Rep 7:8329.
348 19. Dharani S, Kim DH, Shanks RM, Doi Y, Kadouri DE. 2018. Susceptibility of colistin-
349 resistant pathogens to predatory bacteria. Res Microbiol 169:52–55.
350 20. Piñeiro SA, Williams HN, Stine OC. 2008. Phylogenetic relationships amongst the
351 saltwater members of the genus Bacteriovorax using *rpoB* sequences and
352 reclassification of *Bacteriovorax stolpii* as Bacteriolyticum stolpii gen. nov., comb. nov.
353 Int J Syst Evol Microbiol 58:1203–1209.
354 21. Kadouri D, O'Toole GA. 2005. Susceptibility of biofilms to *Bdellovibrio bacteriovorus*
355 attack. Appl Environ Microbiol 71:4044–4051.
356 22. Hobley L, Lerner TR, Williams LE, Lambert C, Till R, Milner DS, Basford SM, Capeness MJ,
357 Fenton AK, Atterbury RJ, Harris MATS, Sockett RE. 2012. Genome analysis of a
358 simultaneously predatory and prey-independent, novel *Bdellovibrio bacteriovorus* from
359 the River Tiber, supports in silico predictions of both ancient and recent lateral gene
360 transfer from diverse bacteria. BMC Genomics 13:670.
361 23. Schwudke D, STRAUCH E, KRUEGER M, APPEL B. 2001. Taxonomic studies of predatory
362 Bdellovibrios Based on 16S rRNA Analysis, Ribotyping and the hit Locus and
363 Characterization of Isolates from the Gut of Animals. Syst Appl Microbiol 24:385–394.
364 24. Iebba V, Santangelo F, Totino V, Nicoletti M, Gagliardi A, De Biase RV, Cucchiara S,

Nencioni L, Conte MP, Schippa S. 2013. Higher prevalence and abundance of Bdellovibrio bacteriovorus in the human gut of healthy subjects. PLoS One 8:e61608.

25. Caballero JDD, Vida R, Cobo M, Máiz L, Suárez L, Galeano J, Baquero F, Canton R, Del Campo R. 2017. Individual patterns of complexity in including predator bacteria , over a 1-Year Period 8:e00959-17.

26. Shatzkes K, Singleton E, Tang C, Zuena M, Shukla S, Gupta S, Dharani S, Onyile O, Rinaggio J, Connell ND, Kadouri DE. 2016. Predatory bacteria attenuate *Klebsiella pneumoniae* burden in rat lungs. MBio 7:1–9.

27. Shanks RMQ, Davra VR, Romanowski EG, Brothers KM, Stella N a, Godboley D, Kadouri DE. 2013. An Eye to a Kill: Using predatory bacteria to control Gram-negative pathogens associated with ocular infections. PLoS One 8:e66723.

28. Romanowski EG, Stella NA, Brothers KM, Yates KA, Funderburgh ML, Funderburgh JL, Gupta S, Dharani S, Kadouri DE, Shanks RMQ. 2016. Predatory bacteria are nontoxic to the rabbit ocular surface. Sci Rep 6:30987.

29. Silva PHF, Oliveira LFF, Cardoso RS, Ricoldi MST, Figueiredo LC, Salvador SL, Palioto DB, Furlaneto FAC, Messora MR. 2019. The impact of predatory bacteria on experimental periodontitis. J Periodontol https://doi.org/10.1002/JPER.18-0485.

30. Martínez V, Herencias C, Jurkevitch E, Prieto MA. 2016. Engineering a predatory bacterium as a proficient killer agent for intracellular bio-products recovery: The case of the polyhydroxyalkanoates. Sci Rep 6:24381.

31. Saralegui C, Ponce-Alonso M, Pérez-Viso B, Moles Alegre L, Escribano E, Lázaro-Perona F, Lanza VF, de Pipaón MS, Rodríguez JM, Baquero F, del Campo R. 2020. Genomics of *Serratia marcescens* isolates causing outbreaks in the same pediatric unit 47 years

388 apart: position in an updated phylogeny of the species. Front Microbiol 11:1–15.

389 32. Pérez-Viso B, Hernández-García M, Ponce-Alonso M, Morosini MI, Ruiz-Garbajosa P, del
390 Campo R, Cantón R. 2020. Characterization of carbapenemase-producing *Serratia*
391 *marcescens* and whole-genome sequencing for plasmid typing in a hospital in Madrid,
392 Spain (2016–18). J Antimicrob Chemother 1–7.

393 33. López-Causapé C, de Dios-Caballero J, Cobo M, Escribano A, Asensio Ó, Oliver A, Del
394 Campo R, Cantón R, Solé A, Cortell I, Asensio O, García G, Martínez MT, Cols M, Salcedo
395 A, Vázquez C, Baranda F, Girón R, Quintana E, Delgado I, de Miguel MÁ, García M, Oliva
396 C, Prados MC, Barrio MI, Pastor MD, Olveira C, de Gracia J, Álvarez A, Escribano A,
397 Castillo S, Figuerola J, Togores B, Oliver A, López C, de Dios Caballero J, Tato M, Máiz L,
398 Suárez L, Cantón R. 2017. Antibiotic resistance and population structure of cystic
399 fibrosis *Pseudomonas aeruginosa* isolates from a Spanish multi-centre study. Int J
400 Antimicrob Agents 50:334–341.

401 34. García-Castillo M, Del Campo R, Morosini MI, Riera E, Cabot G, Willems R, Van Mansfeld
402 R, Oliver A, Cantón R. 2011. Wide dispersion of ST175 clone despite high genetic
403 diversity of carbapenem-nonsusceptible *Pseudomonas aeruginosa* clinical strains in 16
404 Spanish hospitals. J Clin Microbiol 49:2905–2910.

405 35. Li H. 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics
406 34:3094–3100.

407 36. Rutherford K, Parkhill J, Crook J, Horsnell T, Rice P, Rajandream M-A, Barrell B. 2000.
408 Artemis: sequence visualization and annotation. Bioinformatics 16:944–945.

409 37. Liu Y-Y, Lin J-W, Chen C-C. 2019. cano-wgMLST_BacCompare: A bacterial genome
410 analysis platform for epidemiological investigation and comparative genomic analysis.

411 Front Microbiol 10:1687.

412 38. Letunic I, Bork P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new
413 developments. Nucleic Acids Res 47:W256–W259.

414 39. Herencias C, Prieto MA, Martínez V. 2017. Determination of the predatory capability of
415 *Bdellovibrio bacteriovorus* HD100. Bio-protocol 7:2–10.

416 40. Lambert C, Socket RE. 2008. Laboratory maintenance of Bdellovibrio. Curr Protoc
417 Microbiol 1–13.

418 41. Rendulic S, Jagtap P, Rosinus A, Eppinger M, Baar C, Lanz C, Keller H, Lambert C, Evans
419 KJ, Goesmann A, Meyer F, Sockett RE, Schuster SC. 2004. A predator unmasked: life
420 cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689–92.

421 42. Jurkevitch E, Minz D, Ramati B, Barel G. 2000. Prey range characterization, ribotyping,
422 and diversity of soil and rhizosphere Bdellovibrio spp. isolated on phytopathogenic
423 bacteria. Appl Environ Microbiol 66:2365–2371.

424 43. STARR MPASRJ. 1971. The Bdellovibrios. Annu Rev Microbiol 25:649–678.

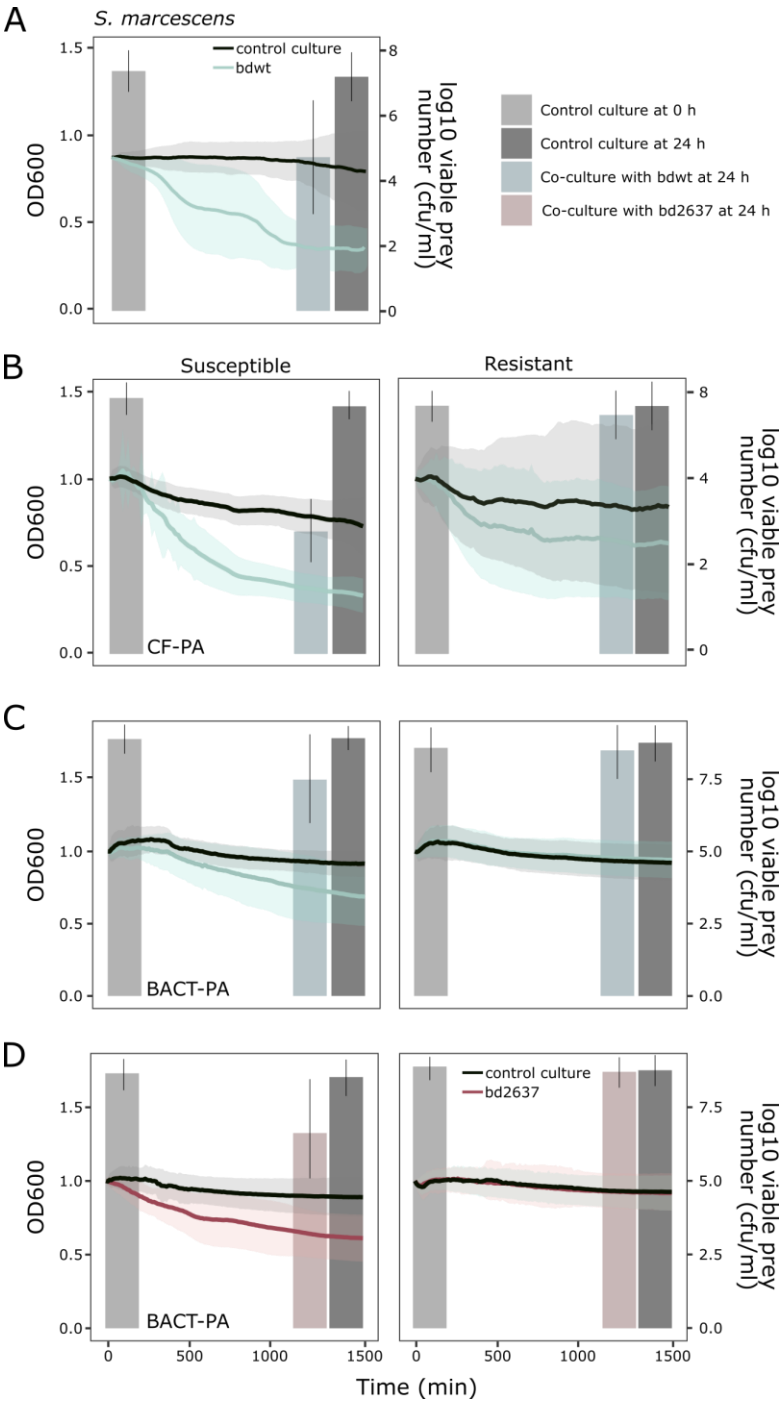
425 44. Westergaard JM, Kramer TT. 1977. Bdellovibrio and the intestinal flora of vertebrates.
426 Appl Environ Microbiol 34:506–511.

427 45. Bonfiglio G, Neroni B, Radocchia G, Marazzato M, Pantanella F, Schippa S. 2020. Insight
428 into the Possible Use of the Predator *Bdellovibrio bacteriovorus* as a Probiotic. Nutrients
429 12.

430 46. Fernández-Olmos A, García-Castillo M, María Alba J, Morosini MI, Lamas A, Romero B,
431 Galán JC, Del Campo R, Cantón R. 2013. Population structure and Antimicrobial
432 susceptibility of both nonpersistent and persistent *Pseudomonas aeruginosa* isolates
433 recovered from cystic fibrosis patients. J Clin Microbiol 51:2761–2765.

434 47. Rogosky AM, Moak PL, Emmert E a B. 2006. Differential predation by *Bdellovibrio*
435 *bacteriovorus* 109J. Curr Microbiol 52:81–5.
436 48. Markelova NY. 2010. Predacious bacteria, Bdellovibrio with potential for biocontrol. Int
437 J Hyg Environ Health 213:428–431.
438 49. Knoll M, Hamm TM, Wagner F, Martinez V, Pleiss J. 2009. The PHA Depolymerase
439 Engineering Database: A systematic analysis tool for the diverse family of
440 polyhydroxyalkanoate (PHA) depolymerases. BMC Bioinformatics 10:89.
441 50. Potter SC, Luciani A, Eddy SR, Park Y, Lopez R, Finn RD. 2018. HMMER web server: 2018
442 update. Web Serv issue Publ online 46.
443 51. Sydney N, Swain MT, So JMT, Hoiczyk E, Tucker NP, Whitworth DE. 2021. The Genetics
444 of Prey Susceptibility to Myxobacterial Predation: A Review, Including an Investigation
445 into *Pseudomonas aeruginosa* Mutations Affecting Predation by *Myxococcus xanthus*.
446 Microb Physiol 1–10.
447
448

449 FIGURES



450

451 **Fig 1. Predation susceptibility of prey collections on hepes buffer.** Monitoring of OD_{600} during

452 24 h of predation and quantification of viable prey number. A) Predation of *B. bacteriovorus*

453 109J upon *S. marcescens* collection (individual predation curves in Fig S1). B) Predation of *B.*

454 *bacteriovorus* 109J upon CF-PA collection (Individual predation curves in Fig S2), C) predation

455 of *B. bacteriovorus* 109J upon BSI-PA collection (Individual predation curves in Fig S3) and D)
456 predation of bd2637 mutant upon BSI-PA collection (Individual predation curves in Fig S6).
457 OD_{600} was measured every 10 min and lines represent the mean of 3 biological replicates, and
458 the shaded area indicates standard error of the mean. Bars represent the means of 3
459 independent viable prey quantification and error bars represent the standard error of the
460 mean. Left and right panels represent the average of susceptible and resistant preys,
461 respectively, of each collection.
462

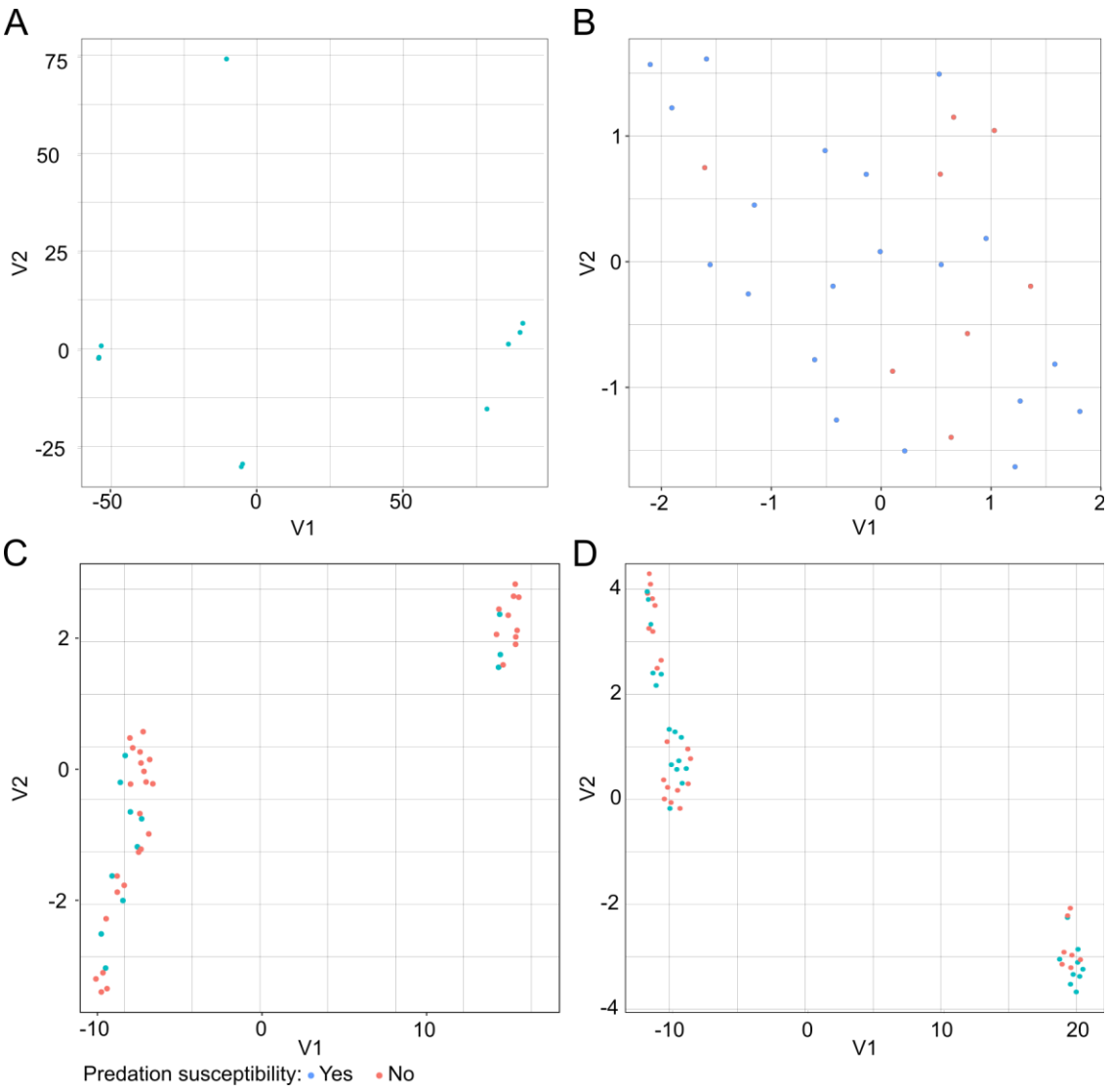


Fig 2. UMAP visualization of the association of predation susceptibility and antibiotic resistant profile of prey cells. A) *S. marcescens* and *B. bacteriovorus* 109J, B) CF-*P. aeruginosa* and *B. bacteriovorus* 109J, C) BACT-*P. aeruginosa* and *B. bacteriovorus* 109J; and D) BACT-*P. aeruginosa* and Bd2637 *B. bacteriovorus* data collections.

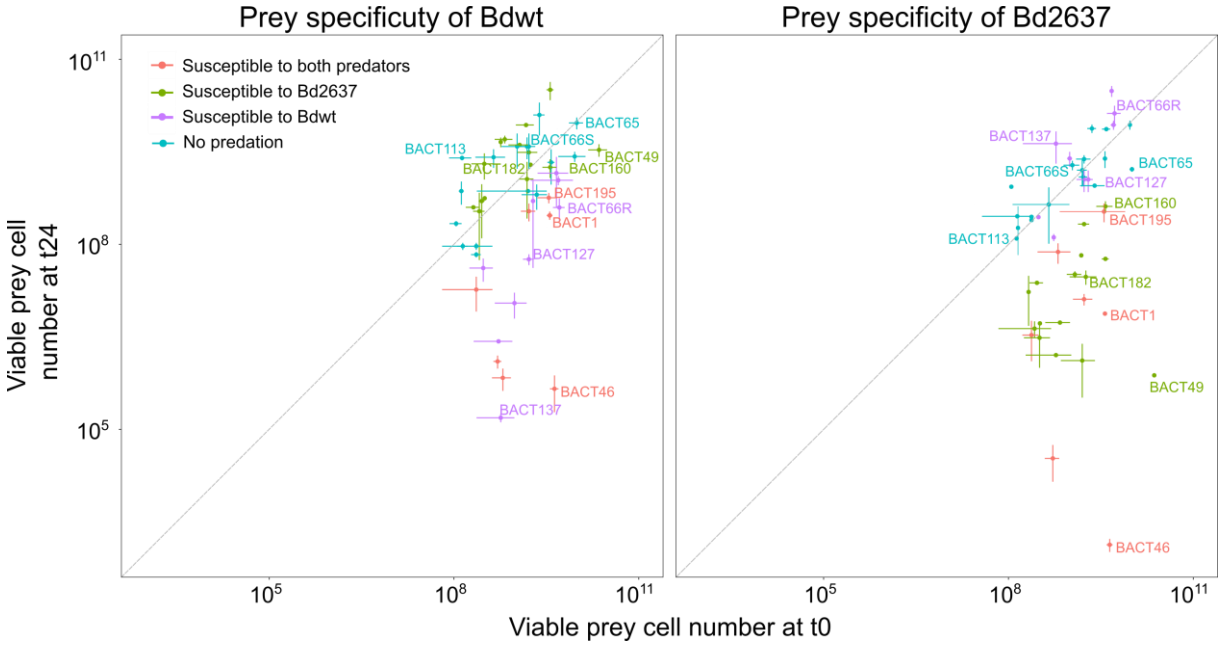
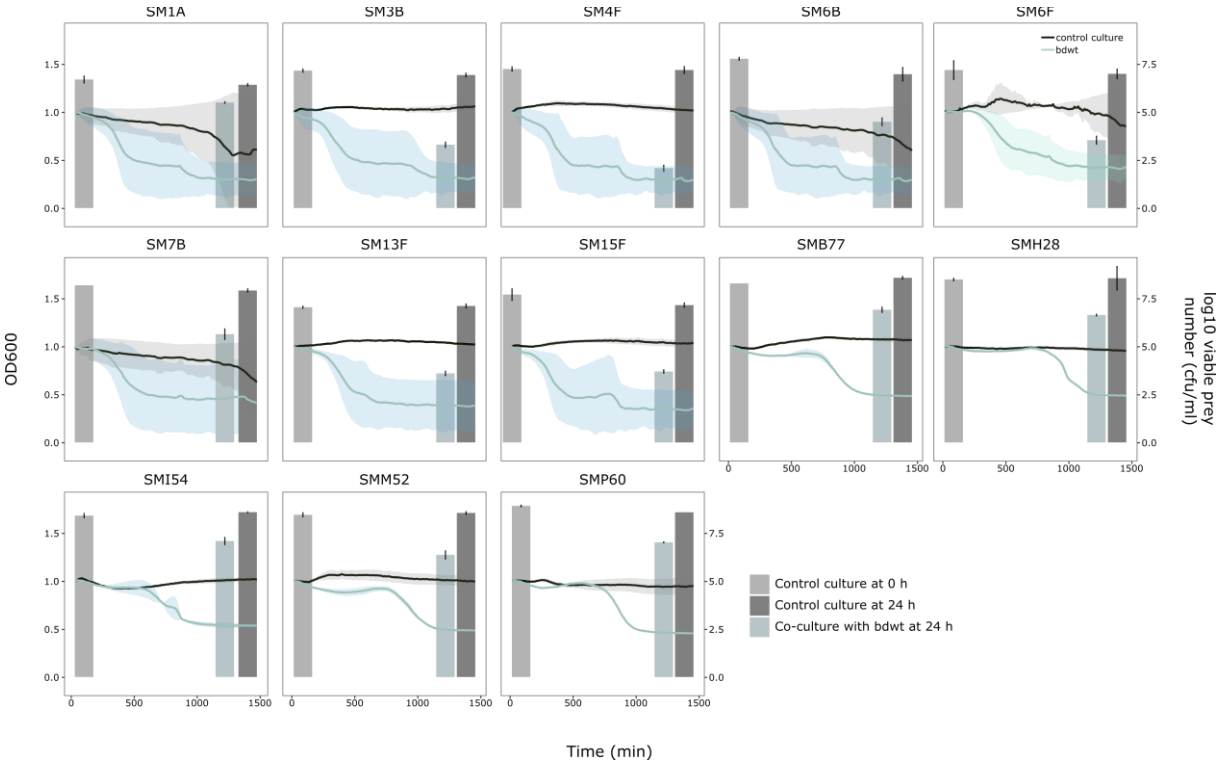


Fig 3. Prey specificity of predators among BACT-*P. aeruginosa* collection. Relationship between the viable prey number at the end (t24) and the beginning (t0) of the predation event. Data points below the grey line represent the susceptible preys for *B. bacteriovorus* 109J and Bd2637 *B. bacteriovorus*.

485 **SUPPLEMENTARY MATERIAL**

486



487

488 **Fig S1. Predation susceptibility of S. marcescens prey collection by *B. bacteriovorus* 109J.**
489 OD_{600} was measured every 10 min and lines represent the mean of 3 biological replicates, and
490 the shaded area indicates standard error of the mean. Bars represent the means of 3
491 independent viable prey quantification and error bars represent the standard error of the
492 mean.

493

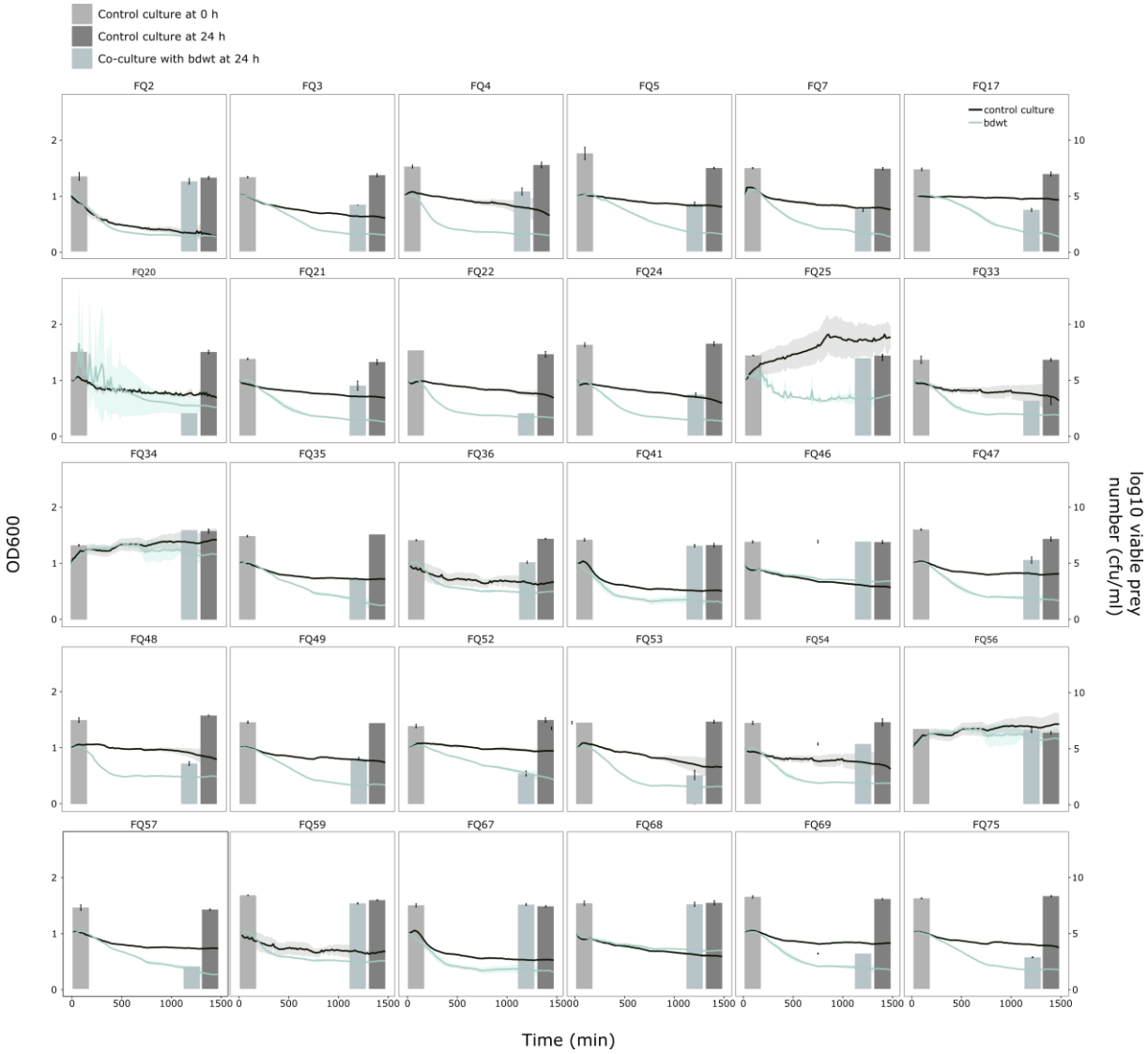


Fig S2. Predation susceptibility of Cf-PA prey collection by *B. bacteriovorus* 109J. OD_{600} was measured every 10 min and lines represent the mean of 3 biological replicates, and the shaded area indicates standard error of the mean. Bars represent the means of 3 independent viable prey quantification and error bars represent the standard error of the mean.

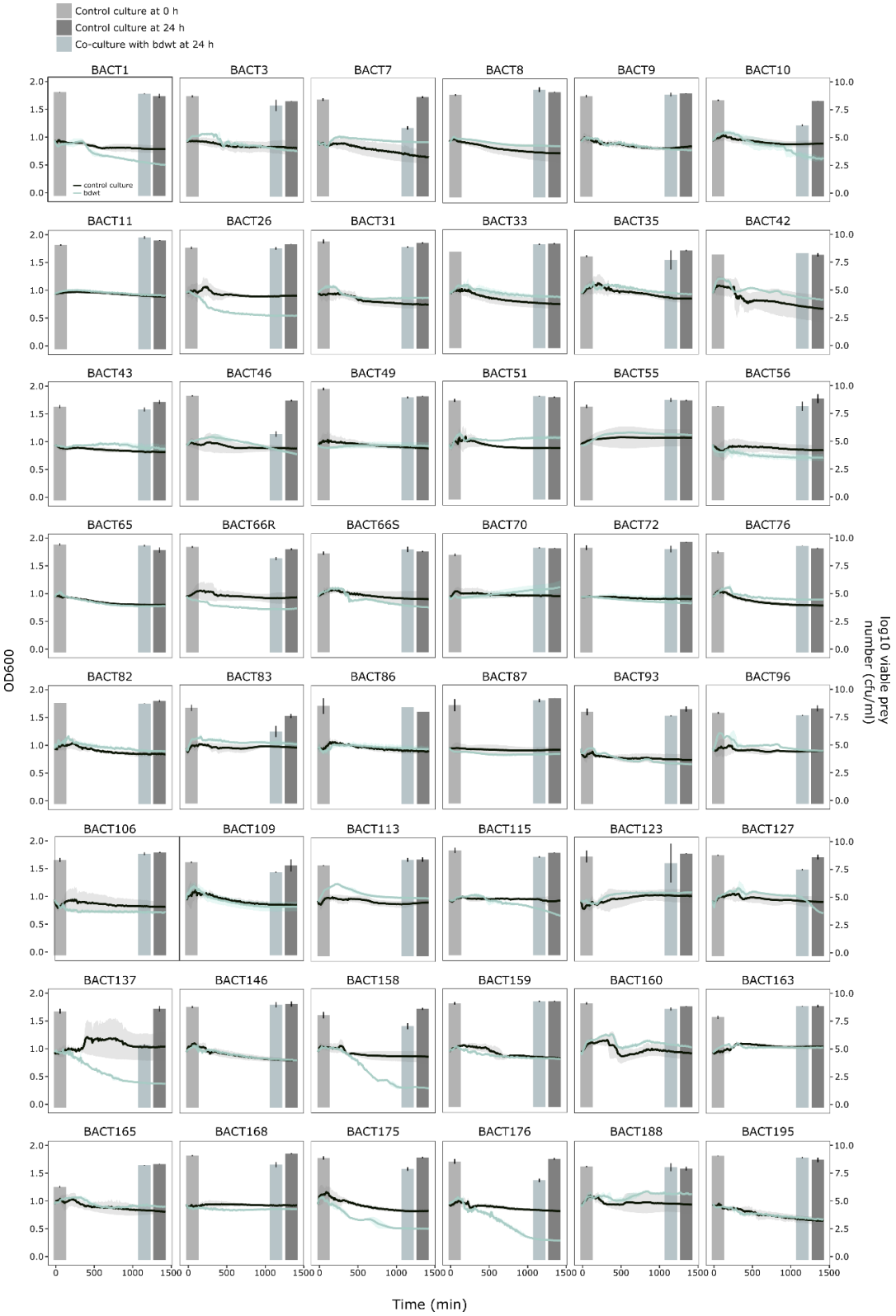
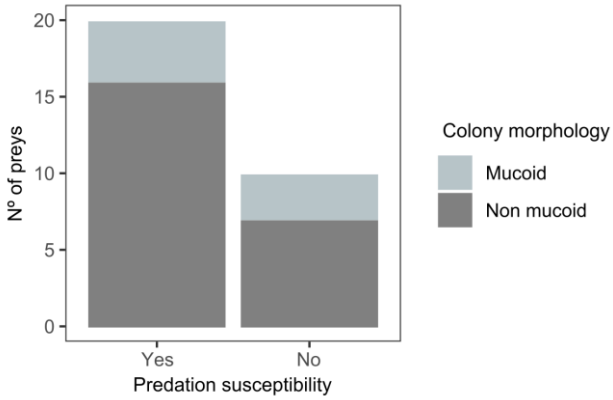


Fig S3. Predation susceptibility of BSI-PA prey collection by *B. bacteriovorus* 109J. OD_{600} was measured every 10 min and lines represent the mean of 3 biological replicates, and the shaded area indicates standard error of the mean. Bars represent the means of 3 independent viable prey quantification and error bars represent the standard error of the mean.



504

505 **Fig S4. Correlation of morphotypes with predation susceptibility.** Number of preys belonged
506 to each morphotype from CF-PA collection.

507

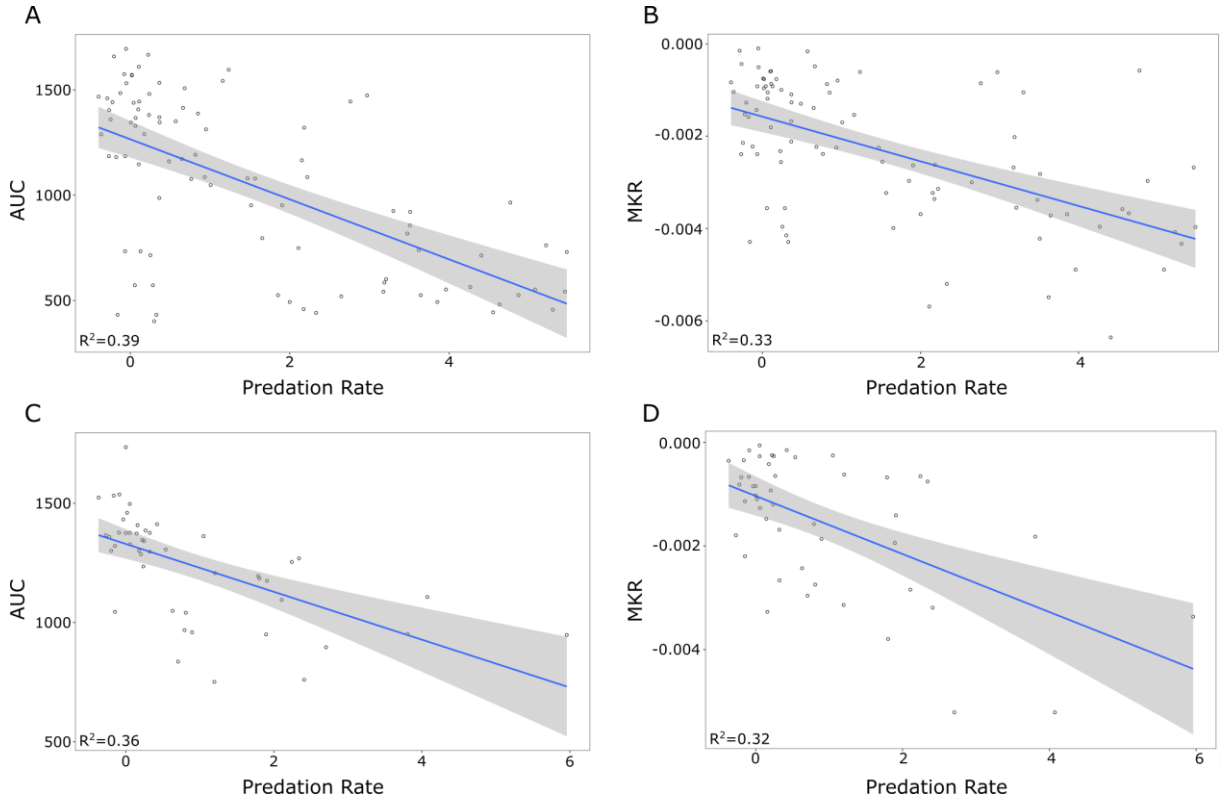


Fig S5. Correlation between kinetic parameter and predation rate of *B. bacteriovorus* 109J (A and B) and the mutant strain bd2637 (C and D). AUC: Area under de curve; MKR: Maximun killing rate.

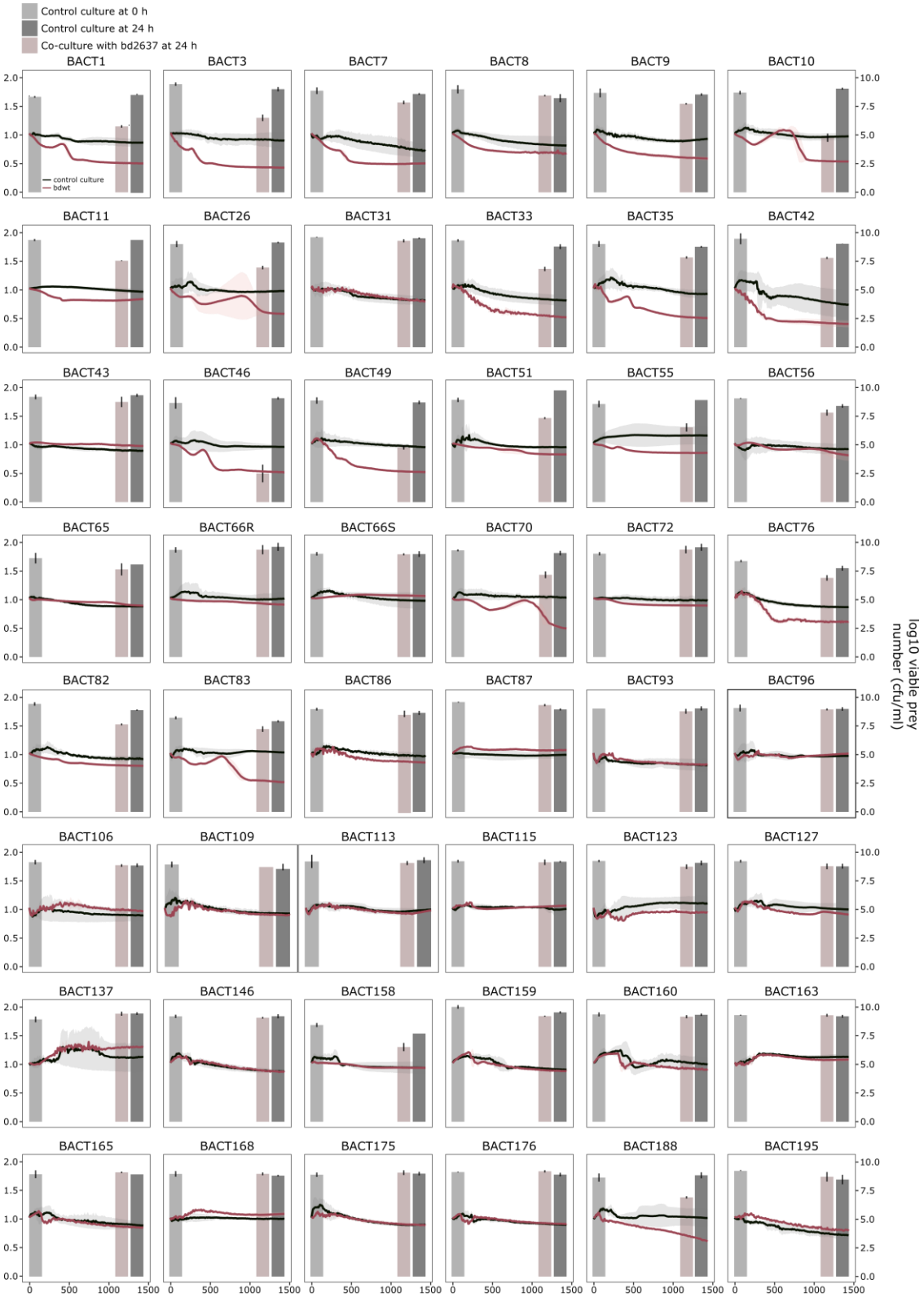


Fig S6. Predation susceptibility of BSI-PA prey collection by bd2637 derivative. OD_{600} was measured every 10 min and lines represent the mean of 3 biological replicates, and the shaded

524 area indicates standard error of the mean. Bars represent the means of 3 independent viable
525 prey quantification and error bars represent the standard error of the mean.
526

527

528 **Table S1. Predation susceptibility analysis of the prey clinical isolates used as prey.**
529

530 **Table S2. Antibiotic resistance profile of the clinical isolates used as prey.**

531

532 **Table S3. Kinetic parameters of predation experiments with *B. bacteriovorus* 109J and**
533 **Bd2637 mutant strain**
534

535 **Table S4. Chromosomal mutations accumulated during gene deletion.**

Gene	Gene Product	Type	Position[1]	Reference[2]	Alternative	Effect
Bd2637	Scl-PHA depolymerase	Insertion	1.948.957	G	GAT	--
Intergenic	-	Deletion	1.043.270	ACTTCTT	A	-

536 [1]Position relative to the chromosomal coordinate. [2]Reference genome is *B. bacteriovorus* 109J
537 (deposited in Sequence Read Archive [SRA], BioProject ID: PRJNA723206). Scl-PHA: short-
538 chain length polyhydroxyalkanoate.
539