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APPLIED MICROBIAL AND CELL PHYSIOLOGY

Bdellovibrio's prey-independent lifestyle is fueled by amino acids as a carbon source

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Abstract

Identifying the nutritional requirements and growth conditions of microorganisms is crucial for determining their applicability in industry and understanding their role in clinical ecology. Predatory bacteria such as *Bdellovibrio bacteriovorus* have emerged as promising tools for combating infections by human bacterial pathogens due to their natural killing features. *Bdellovibrio's* lifecycle occurs inside prey cells, using the cytoplasm as a source of nutrients and energy. However, this lifecycle supposes a challenge when determining the specific uptake of metabolites from the prey to complete the growth inside cells, a process that has not been completely elucidated. Here, following a model-based approach, we illuminate the ability of *B. bacteriovorus* to replicate DNA, increase biomass, and generate adenosine triphosphate (ATP) in an amino acid-based rich media in the absence of prey, keeping intact its predatory capacity. In this culture, we determined the main carbon sources used and their preference, being glutamate, serine, aspartate, isoleucine, and threonine. This study offers new insights into the role of predatory bacteria in natural environments and establishes the basis for developing new *Bdellovibrio* applications using appropriate metabolic and physiological methodologies.

Key points

- *Amino acids support axenic lifestyle of Bdellovibrio bacteriovorus.*
- *B. bacteriovorus preserves its predatory ability when growing in the absence of prey.*

Keywords *Bdellovibrio bacteriovorus* · Nutritional requirements · Axenic lifestyle · Metabolic modelling

Introduction

A complete understanding of the physiological and nutritional requirements that enable optimal cell growth plays an important role in the use of microorganisms in biotechnological, industrial, and medical applications. In recent years, there has been an increase in new approaches to efficiently growing microorganisms by taking advantage of new knowledge of their physiology (Chubukov et al. 2014; Monk et al.

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2014). *Bdellovibrio bacteriovorus* is a gram-negative predatory bacterium belonging to the group of *Bdellovibrio* and like organisms (BALOs) that prey on other gram-negative bacteria. This species is ubiquitous, given that it can be found in soil, aquatic environments, and human commensal microbiota (Herencias et al. 2020a). Through a periplasmic predation strategy, *Bdellovibrio* uses the nutrients in its prey's cytoplasm as carbon and energy sources for growth. Once the prey is exhausted, *Bdellovibrio* septates into its progeny and finally lyses the prey cell to begin another attack (Fig. 1a). Under certain conditions (limited nutrients/prey), certain *Bdellovibrio* strains can change their lifestyle strategy and develop as prey-independent cells (Hobley et al. 2012). These *Bdellovibrio* variants can grow in the absence of prey bacteria, and their genotypic characterisation has revealed that they possess various mutations in genes related to the transduction of type IV pili (T4P) interactions, critical for prey recognition and attachment (Seidler and Starr 1969; Barel and Jurkevitch 2001; Evans et al. 2007; Chang et al. 2011; Morehouse et al. 2011; Roschanski et al. 2011; Capeness et al. 2013; Kaplan et al. 2023).

Derived from its lifestyle, multiple applications of the predators in various fields have been proposed, such as biocontrol agents (food preservation and water treatment), living antibiotics (to combat multiresistant pathogens), and hydrolytic enzyme producers (Herencias et al. 2020a; Lai et al. 2023).

Several biochemical studies have highlighted aspects of the energetic metabolism of *Bdellovibrio* using diverse substrates such as amino acids and RNA (Hespell et al. 1973; Hespell et al. 1974; Hespell and Odelson 1978; Rosson and Rittenberg 1979; Nelson and Rittenberg 1981; Rosson and Rittenberg 1981). Analysis of the *Bdellovibrio* genome revealed the presence of complete biosynthetic and degradation pathways for fatty acids; however, the lack of a phosphotransferase system makes the use of carbohydrates unlikely (Rendulic et al. 2004; Herencias et al. 2020b). More recent studies have examined the possibility of growing predator cells in a rich medium, offering new biotechnological perspectives (Sathyamoorthy et al. 2021). Cell survival is minimally altered during prey starvation in the attack phase (Sathyamoorthy et al. 2021), and there is a slight increase in cell size and stimulation of protease secretion (Dwidar et al. 2017).

Currently, the precise nutritional requirements that allow for the growth of these predators are not entirely elucidated, mainly due to this organism's host-dependent nature (Lai et al. 2023). Analysing which metabolites from the prey cells are taken up during intraperiplasmic growth is a challenge, given the vast repertoire of molecules contained in the prey cell.

We have reported a genome-scale metabolic model of this predatory bacterium (*i*CH457) to characterise and predict the metabolic potential and behavior of the biphasic

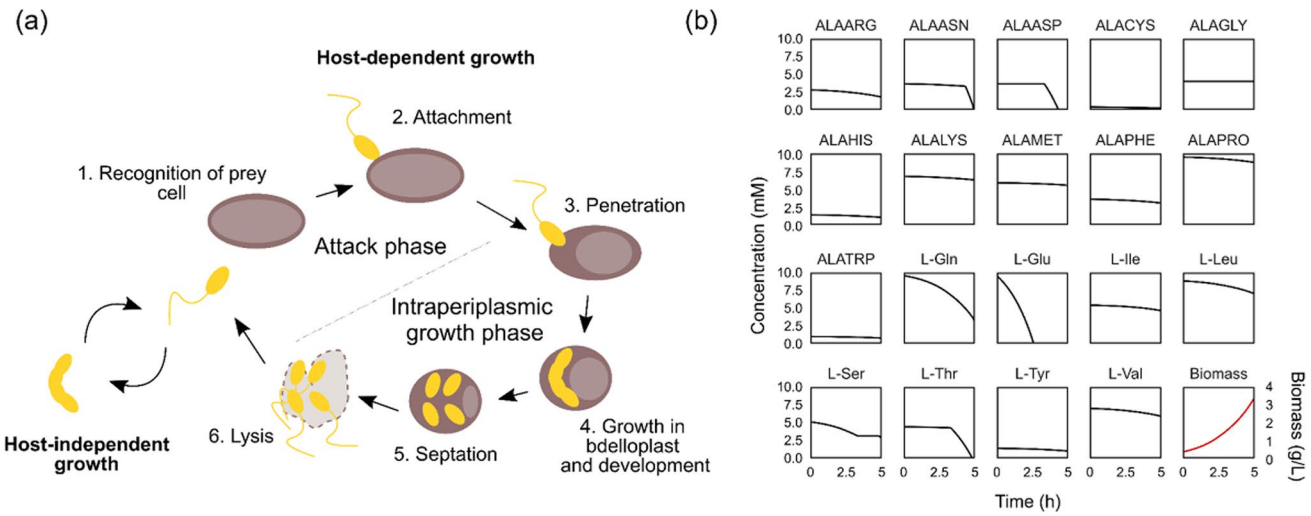

Fig. 1 Representation of *B. bacteriovorus* lifecycle and metabolic simulation of its growth capacity. **a)** During host-dependent growth, *B. bacteriovorus* cells follow these steps: 1) Prey recognition: *Bdellovibrio* moves towards prey-rich regions. 2) Attachment: *Bdellovibrio* anchors to the host cell, which leads to the infection. 3) Penetration: *Bdellovibrio* enters the periplasm of the prey cell. 4) Growth in bdelloplast and development: the prey appears rounded due to cell wall modification, and *Bdellovibrio* grows in the periplasm and replicates its DNA. *Bdellovibrio* uses the prey biomolecules as a source of nutrients. 5) Septation: *Bdellovibrio* septates when resources become limited and matures into new individual attack phase cells. 6) Lysis: mature attack-phase cells lyse the cell wall of the bdelloplast, are released in the environment and start a new search for fresh prey. The complete host-dependent cycle takes approximately 4 h. During host-independent growth (left), *Bdellovibrio* cells elongate into filaments using the nutrients available in the environment to grow and finally sept into several daughter cells. **b)** Analysis of the growth capacity of *B. bacteriovorus* using the *i*CH457 model. Each plot represents the prediction of amino acid consumption present in the rich media. The red line indicates the prediction of biomass production

lifestyle of *B. bacteriovorus* (Herencias et al. 2020b). Our model simulations have consistently postulated the growth capacity of *Bdellovibrio* in a rich amino acid medium in the absence of prey, based on its metabolic competencies.

The present study aimed to determine the metabolic capabilities of the host-dependent *B. bacteriovorus* 109J, a laboratory reference strain, in a nutrient-rich but prey-free medium, and the uptake of metabolites during cellular growth. A better understanding of how these bacteria grow and their physiological and metabolic requirements will provide insight into their role in various microbial environments and increase the possibility of producing and applying predators rationally and safely in industry and clinical practice.

Materials and methods

In silico growth simulations

Biomass production and amino acids consumption were calculated using the dynamic Flux Balance Analysis (dFBA) of the COBRA toolbox 3.0 (Heirendt et al. 2018) in the environment MATLAB R2022a (MathWorks, Natick, MA, USA) using the Gurobi Solver (v9.5.2, Gurobi Inc.). Biomass was set as the objective function and the bounds of the exchange fluxes were set as the rich in silico medium as described previously (Herencias et al. 2020b). Amino acids exchange reactions were set as "substrateRxns", and "initConcetrations" of amino acid or dipeptides were based on Lysogeny Broth (Sezonov et al. 2007). Since L-alanine is supplied in the form of dipeptides, the initial concentration of L-alanine was set in 0.001 mM, as well as the other unsupplied dipeptides, to avoid being set unlimited for the function dFBA. Amino acids and dipeptides transporters are included in the metabolic model, based on previous studies (Herencias et al. 2020b).

Strains, media, and growth conditions

B. bacteriovorus 109J (NCBI Reference Sequence: NZ_CP007656) was routinely grown at 30 ºC in Hepes buffer (25 mM Hepes amended with 2 mM $CaCl_2{\cdot}2H_2O$ and 3 mM $MgCl_2{\cdot}3H_2O$, pH 7.8) or DNB liquid medium (consisting of 0.8 g L^{-1} NB (Difco™) (supplemented with 2 mM $CaCl_2{\cdot}2H_2O$ and 3 mM $MgCl_2{\cdot}3H_2O$) with *Pseudomonas putida* KT2440 as prey (Herencias et al. 2017; Saralegui et al. 2022). To remove the prey, the co-cultures were filtered twice through a 0.45 µm filter (Sartorius). The axenic cultivation of *Bdellovibrio* was carried out in PYE10 (10 g L^{-1} peptone and 10 g L^{-1} yeast extract) medium. CAV defined medium is composed of 200 µM of each amino acid: phenylalanine, glutamate, aspartate, threonine, serine, glycine, proline, isoleucine, leucine, valine and alanine. CAV medium was supplemented with a solution of trace elements (composition 1000 × 2.78 $FeSO_4{\cdot}7H_2O$ g L^{-1}, 1.98 $MnCl_2{\cdot}4H_2O$ g L^{-1}, 2.81 $CoSO_4{\cdot}7H_2O$ g L^{-1}, 1.47 $CaCl_2{\cdot}2H_2O$ g L^{-1}, 0.17 $CuCl^2{\cdot}2H^2O$ g L^{-1}, 0.29 $ZnSO_4{\cdot}7H_2O$ g L^{-1}), 5 µM of KH_2PO_4, 5 µM of K_2HPO_4 and 5 µM of $NHCO_3$. All these compounds are suspended in Hepes buffer.

B. bacteriovorus viability calculation

Predator viability was counted as plaque forming units per milliliter (pfu mL^{-1}). It was calculated from a culture performing serial dilution from 10^{-1} to 10^{-7} in Hepes buffer and developing on the lawn of prey after 48–72 h of incubation at 30 °C by using the double layer method as described previously (Herencias et al. 2017; Saralegui et al. 2022). Briefly, 0.1 mL of the appropriate culture dilution was mixed with an additional 0.5 mL of prey cell suspension of *P. putida* KT2440 (previously pre-grown in NB and prepared in Hepes buffer OD_{600} 10) and 4 mL of DNB 0.7 % agar. The final mix was vortexed and plated on DNB solid medium (1.5 % agar).

Biomass calculations

Cellular biomass, expressed in grams of cell dry weight (g CDW) per liter, was determined gravimetrically as previously reported (Martínez et al. 2013). Briefly, 10 ml of culture medium was centrifuged for 15 min at 13000 × *g* at 4 °C. Cell pellets and the supernatants were separated, subsequently dried at -80 ºC for 24 h, lyophilized, and finally weighed. The biomass calculation was obtained from the pellet weight.

Measurements of ATP intracellular levels

Intracellular ATP levels were determined by an ATP bioluminescence assay kit (ATP Biomass Kit HS, BioThema) according to the manufacturer's instructions. To measure the intracellular ATP, 1 mL of *B. bacteriovorus* cells was centrifuged for 15 min at 13,000 × *g* and 4 °C and the pellet was suspended in 1 mL of saline solution (0.85 % NaCl). For each condition, four different experiments were carried out and two technical replicates were measured. To normalize the ATP intracellular values, the number of viable predator cells was also measured by the double-layer method.

Quantification of *B. bacteriovorus* genome number

The abundance of the predator (number of genomes) was estimated by quantitative PCR (qPCR). A DNA fragment of 121 bp located in the coding region of the housekeeping gene *bd2400* (Dori-Bachash et al. 2008) was amplified using the oligonucleotides Bd2400-1 (5´-GCGACTCCA GAACAGCAGATT) and Bd2400-2 (5´-GAATCCGCG

GACTGCATTGTA) (Dori-Bachash et al. 2008). qPCR was performed using the SYBR Green (LightCycler® 480 SYBR Green I Master) technology in a LightCycler 480 Real-Time PCR system. Samples were directly analyzed from the culture (containing either prey and predator or predator alone), without DNA extraction. For the calibration curve, genomic DNA was purified from a filtered co-culture of *B. bacteriovorus* 109J using the IllustraTM bacteria genomicPrep Mini Spin Kit (GE Healthcare) following the instructions of the manufacturers. For the measurements, 200 µL of each sample was collected and stored at -20 °C until analysis. Samples were initially denatured by heating at 95 °C for 5 min, followed by 45 cycles of amplification (95 °C, 10 s; test annealing temperature, 60 °C, 10 s; elongation and signal acquisition, 72 °C, 10 s). For quantification of the fluorescence values, a calibration curve was made using serial dilution from 5 to $5{\cdot}10^{-7}$ ng of *B. bacteriovorus* 109J genomic DNA sample. qPCR was performed with triplicate samples from three independent biological experiments. The negative control was achieved with genomic DNA from *P. putida* KT2440 as a template. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method and the genome number per mL can be calculated as follows (Livak and Schmittgen 2001; Van Essche et al. 2009): n° of genomes of Bd = (ng of DNA in the sample by qPCR)/(weight of the amplicon), being the weight of the amplicon of $1.24{\cdot}10^{-10}$ ng. The calculations of concentration (genome mL^{-1}) consider that the genome of the predator contains one copy of the *bd2400* gene. Genes covering the whole genome were selected to validate the qPCR method and are listed in Table S2 and Table S3.

Correlation between viable cells and genome number

To correlate qPCR data with viable *Bdellovibrio* cells, we prepared co-cultures with the predator and *P. putida* KT2440 as prey at different predator-to-prey ratios (1:0.1, 1:1, and 1:10) and initial prey cell numbers (3×10^8 and 6×10^8). A total of 30 co-cultures were incubated for 24 h at 30 °C with shaking at 250 rpm. Plaque-forming units and prey genome numbers were measured at the beginning and end of the predation assay. The data were then transformed to logarithm as previously described (Ogundero et al. 2022) and used to obtain the following linear regression equation: $y = 0.48 + 1.2\,x$ as shown in Fig. S1, where "y" represents the measured genome number using qPCR assay and "x" is the calculated viable predator cells.

Amplification of DNA and hit locus sequencing

PCR amplifications were performed in the buffer recommended by the manufacturer adding 0.05 µg of template DNA, 1 U of Phusion DNA polymerase and 0.4 µg of each deoxynucleotide triphosphate. Conditions for amplification were chosen according to the GC content of the oligonucleotides used, provided by the manufacturer (Sigma-Aldrich). DNA fragments were purified by standard procedures using the Gene Clean Turbo Kit (MP Biomedicals). PCR products were purified using the High Pure Purification Kit (Roche Applied Science). The oligonucleotides used to amplify and sequence the different hit-related genes are listed in Table S4.

Whole genomic sequencing

Genomic DNAs of *B. bacteriovorus* 109J incubated under axenic conditions in HEPES buffer and PYE10 after 24 h were extracted with a PureLink™ Genomic DNA Mini Kit (Invitrogen from Thermo Fisher Scientific). For whole genomic sequencing, the final concentration was adjusted to 50 ng/µL using a NanoPhotometer® bioNova spectrometer (Implen). Genome sequencing was carried out by Plasmidsaurus using Oxford Nanopore technology. Whole Genome Shotgun BioProject has been deposited at DDBJ/ENA/GenBank under the ID PRJNA1104159. The Sequence Read Archive (SRA) data was submitted under accessions SAMN41069971 and SAMN41069972 for HEPES and PYE10 conditions, respectively. Mapping and single nucleotide polymorphisms (SNP) calling were performed by Geneious Prime version 2020.0.4 created by Biomatters (https://www.geneious.com) using *B. bacteriovorus* 109J (NZ_CP007656) as the reference genome.

Analysis of extracellular metabolites by untargeted GC-TOF–MS

The supernatants of the cultures were collected, frozen at -80 °C and lyophilized. All GC identifications were based on retention times and/or comparisons with commercially available standards. Moreover, the National Institute of Standards and Technology (NIST) (Neto et al. 2016) Mass Spectra Library based on a pre-defined matching criterium (similarity index ≥ 70 % (Scheubert et al. 2017) against mass spectral libraries was used.

Before analyses, samples were derivatized to increase the volatility of polar metabolites. For this purpose, 5–10 mg of the lyophilized supernatant, 300 µL of pyridine and 200 µL of BSTFA (with 1 % TMCS) were added to each sample and heated with stirring at 70 °C for 1 h. Subsequently, the reaction mixture was transferred to the GC autosampler vials for further GC-TOF–MS analyses (Chang and Ho 2014).

Agilent 7890A Gas Chromatography (Agilent Technologies) coupled to Waters Micromass GCT Premier Mass Spectrometer (coupled to COMBI-PAL-GC (EI)) (Waters Corporation, Milford) was used for separation and detection in the GC-TOF–MS setup. A GC column

(ZB-5MSplus, Phenomenex) of 30.0 m × 250 µm × 0.25 µm was used. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. The split ratio for the injector was set to 1 : 10, with a total injection volume of 2 µL. Front inlet and ion source temperatures were both kept at 270 °C. Oven temperature was set to equilibrate at 60 °C for 1 min, before initiation of sample injection and GC run. After sample injection, the oven temperature was increased at a rate of 6 °C min^{-1} to 325 °C and held at 325 °C for 3 min. The MS detection was operated in EI mode (70 eV) with a detector voltage of 1900 V. Full scan mode with a mass range of m/z 50–800 was used as the data acquisition method. The metabolite identities will be confirmed by targeted analysis.

Phase-contrast microscopy

To monitor the physiology of *Bdellovibrio* cells, cultures were routinely visualized using a 100× phase-contrast objective and images taken with a Leica DFC345 FX camera.

Statistical analysis

Data sets were analyzed using Prism 6 software (GraphPad Software Inc.). Comparisons between two groups were made using Mann–Whitney-test followed by a no-parametric Krustal-Wallis analysis. The relation between the microbial counting and the genome number was analyzed by Pearson correlation. Data was represented using a R custom script and the "ggplot2" package.

Results

Rich medium supports the growth of *B. bacteriovorus* 109J in the absence of prey

The rational application of predatory bacteria for biotechnological or clinical purposes depends, to a large extent, on the level of understanding of the bacteria's lifecycle and growth requirements. The biphasic lifecycle of *B. bacteriovorus* can complicate its cultivation at a large scale and its consequent applications; however, recent evidence of its metabolic capabilities for axenic lifestyle will facilitate these processes (Herencias et al. 2020b). To provide further insights on this topic, we employed the curated genome-scale model for *B. bacteriovorus* (*i*CH457) to predict cell growth by conducting a dynamic flux balance analysis (dFBA). Combining this approach with the optimization of biomass production under rich medium conditions (rich in silico medium (Herencias et al. 2020b)) it was predicted a specific growth rate of 0.486 h^{-1} (Fig. 1b). Furthermore, we found an interesting hierarchy in the amino acid consumption being glutamate the first carbon source used, followed by glutamine, serine, threonine, and asparagine (Fig. 1b).

The above in silico results strongly argue in favour of the full capacity of *Bdellovibrio* to grow axenically using amino acids as carbon and energy sources. To test this hypothesis, we cultivated the bacteria for 96 h in an amino acid-based media containing peptone and yeast extract (PYE10) and monitored growth parameters such as the viable cell count, the number of genomes, the intracellular ATP levels, and the biomass production. The results showed that while the number of viable predators decreased by 1.32 $\log_{10}$ after 96 h (median log of viable cell number in PYE10 at 0 and 96 h is 9.28 ± 0.25 and 7.97 ± 0.25, respectively), the genome number increased by 0.5 $\log_{10}$ (median log of genome number in PYE10 at 0 and 96 h is 9.23 ± 0.08 and 9.72 ± 0.23, respectively), suggesting that a number of the surviving cells replicated their DNA without compromised viability (Fig. 2a and b). In other words, a subpopulation of *B. bacteriovorus* cells can replicate DNA in PYE10 while others lose viability. The total biomass content and intracellular ATP concentration of the axenically grown *B. bacteriovorus* also increased up to 2.5-fold and 5-fold, respectively, after 24 h (Fig. 2c and d). Under these growth conditions, we obtained a homogenous population that would allow for a reliable and reproducible analysis of their physiological and metabolic states. Unexpectedly, the cells in the axenic culture were significantly larger than those in the control culture in the HEPES buffer (the mean size of PYE-Bd and HEPES-Bd were 1.27 ± 0.5 and 0.65 ± 0.21 µm, respectively; Mann-Whitnee *U*-test, $p < 0.0001$) (Fig. 3), in concordance with previous works measuring cell size under axenic conditions (Dwidar et al. 2017). In contrast, when the predators were inoculated in HEPES buffer, all monitored parameters decreased, indicating cell death.

To confirm the predatory capability of the *B. bacteriovorus* cells after incubation in prey-free PYE10 rich media, we performed predation experiments on *P. putida* KT2440. Our analysis of prey viability after 24 h of predation confirms the killing activity of the axenic *Bdellovibrio* cells, thereby ruling out the possibility of loss of the predatory genotype after cultivation in prey-free conditions (Fig. 3).

The genotype of the wild-type *B. bacteriovorus* 109J was also verified by sequencing the genes associated with the host-independent (HI) phenotype (*bd0108*, *bd3461*, and *bd3464* (Cotter and Thomashow 1992)). Sequences were compared with two HI *Bdellovibrio* mutants, HI18 and HI24 (Prof. Jurkevitch lab collection). No significant mutations were accumulated during the incubation in the absence of prey (Fig. S2). To rule out mutations in other locations, we sequenced the genome of the predator cells after 24 h of axenic incubation in Hepes buffer or PYE10 medium. Comparison of the whole genome sequence of *B. bacteriovorus* 109J growing in co-culture with prey or axenically did not

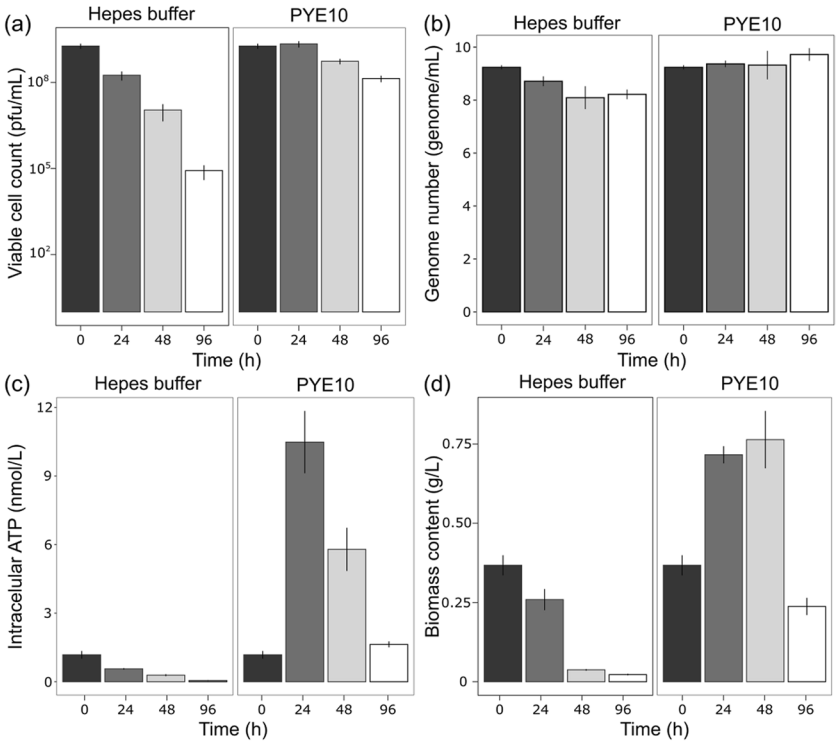

Fig. 2 Growth parameters of *B. bacteriovorus* 109J incubated in the absence of prey for 24 h in HEPES buffer and PYE10 rich. **a)** Viable cell counts of *Bdellovibrio* measured as pfu/mL. **b)** Genome number measured using the *bd2400* housekeeping gene. **c)** Measurement of intracellular ATP levels of *Bdellovibrio* cells**. d)** Total biomass content. Error bars indicate the standard deviation of three biological replicates. Statistical analyses are listed in Table S1

reveal any significant mutations (Fig. S3 and Supplementary Dataset 2).

Overall, these results demonstrated the active metabolism of the wild-type predator grown in PYE10 media, supported by the active DNA replication, the increased biomass content and intracellular ATP concentration, and the maintenance of the predatory capability. Regarding cell size, our data suggest that incubation in PYE10 results in an increase in cell size (Fig. 3a), even though the 109J strains contain a mutational change in the *bd1075* gene related to typical vibrio curvature and shape (Banks et al., 2022).

Analysis of metabolite consumption during *Bdellovibrio* culture in PYE10 medium

To determine the nutrient composition of the PYE10 medium and monitor the uptake of metabolites by *B. bacteriovorus*, we conducted a gas-chromatography/quadrupole time-of-flight (GC-QTOF) analysis. Predator cells were incubated in PYE10 medium; after 96 h, the supernatant was collected and analysed. Amino acids constituted 75 % of all nutrients present and detected in the PYE10 medium (Fig. 4a). In addition, the main non-amino acid metabolites detected in the PYE10 medium were compounds related to secondary metabolism such as butanoic, propanedioic, and gluconic acids. The analysis also revealed relatively high amounts of trehalose, xanthine, and pyranose derivatives and phthalic acid after incubation of the predator cells**.** There were minimal spontaneous variations in the composition of the control medium without predator after 96 h of incubation (Fig. S4 and Supplementary dataset 1), as previously reported (Ames and MacLeod 1985). In contrast, the presence of *Bdellovibrio* drastically altered the composition of the medium, primarily through the consumption of amino acids (Fig. 4b, Fig. S5, and Supplementary Dataset 1). The relative amounts of glutamate, serine, aspartate, isoleucine, and threonine were significantly lower compared with the control culture at the start of the experiment (unpaired *t*-test $pval < 0.02$). The results confirmed the preference consumption of amino acids over the small organic acids detected that were mostly secreted (Fig S4 and Supplementary Dataset 1).

Amino acid-based medium maintains *B. bacteriovorus* metabolically active

A key limitation of the growth rate of bacteria is nutrient concentration. The above results suggest the use of determined amino acids would be sufficient to support *B. bacteriovorus* growth. To validate this hypothesis, we designed the CAV medium, which only contains the amino acids detected in the previous GC-QTOF-MS analysis as carbon and nitrogen sources (see the "Materials and Methods" section). To monitor the growth dynamics of *B. bacteriovorus* in the absence of prey, we incubated the predator cells in the defined CAV medium. The axenic behavior was monitored by measuring the viable cell count, the genome number, the biomass content, and the intracellular ATP concentration. The analysis of these parameters (Fig. 5) revealed the

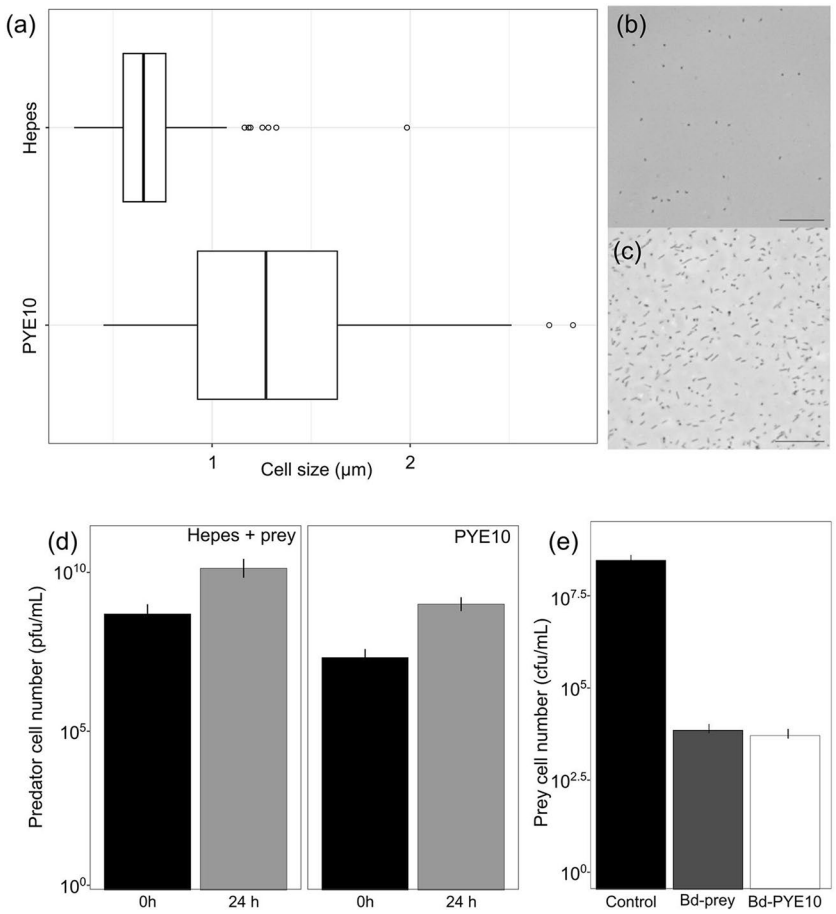

Fig. 3 Morphology and predatory ability of *B. bacteriovorus* after incubations in HEPES or PYE10 medium. Data shown correspond to *Bdellovibrio* cells incubated in PYE10 for 24 h previously. **a)** Boxplot representations of the cell size population in µm using ImageJ software. Vertical lines within boxes indicate median values, left and right hinges correspond to the 25th and 75th percentiles, and whiskers extend to observations within 1.5 times the interquartile range ($n = 150$, Mann–Whitney U test; p-value < 0.01). **b) and c)** Phase-contrast micrography of *Bdellovibrio* incubated in HEPES buffer and PYE10 medium, respectively. Black bar corresponds with 10 µm. **d)** Determination of predator cell counts after 24 h of incubation in co-culture with prey (left panel: HEPES + prey) or axenic incubation in PYE10 medium during 24 h. Error bars indicate the standard deviation of six biological replicates. **e)** Determination of prey viable cell count after predation of *B. bacteriovorus* 109J cells routinely grown with prey in HEPES (Bd-prey) and prey viable number after predation with *Bdellovibrio* grown axenically in PYE10 (Bd-PYE10). Error bars indicate the standard deviation of six biological replicates

maintenance of an active metabolism. There was no increase in the viable cell count or genome number under this condition, but the biomass content increased significantly during the 48 h ($p < 0.03$, Krustal-Wallis t-test). After this point, cells strongly sense starvation, resulting in cell energy depletion and biomass reduction at 96 h.

Discussion

The evolution of biological systems is characterized by their ability to acquire diverse strategies (lifestyles) to adapt to various environments. A significant aspect of this adaptation is the preference for specific carbon and energy sources, which results from metabolic adjustments to the environment. *B. bacteriovorus* has evolved to thrive in the periplasmic space of the prey cell by using the prey cell's cytoplasmic components (Sockett 2009; Lai et al. 2023). Several reports have contributed valuable insights into the energetic metabolism and growth conditions of *Bdellovibrio* (Gadkari and Stolp 1975, 1976); however, there is still a dearth of evidence regarding the specific nutritional requirements of *Bdellovibrio* and preferred carbon source as a predator. This study analysed the carbon and energy requirements necessary to support the growth of *Bdellovibrio*. Understanding these critical factors is vital for potential industrial and medical applications of *Bdellovibrio*.

The bacterial cytoplasm serves as a highly concentrated compartment, containing a significant portion of the cell's macromolecules (30–40 %) and proteins (over 70 %)

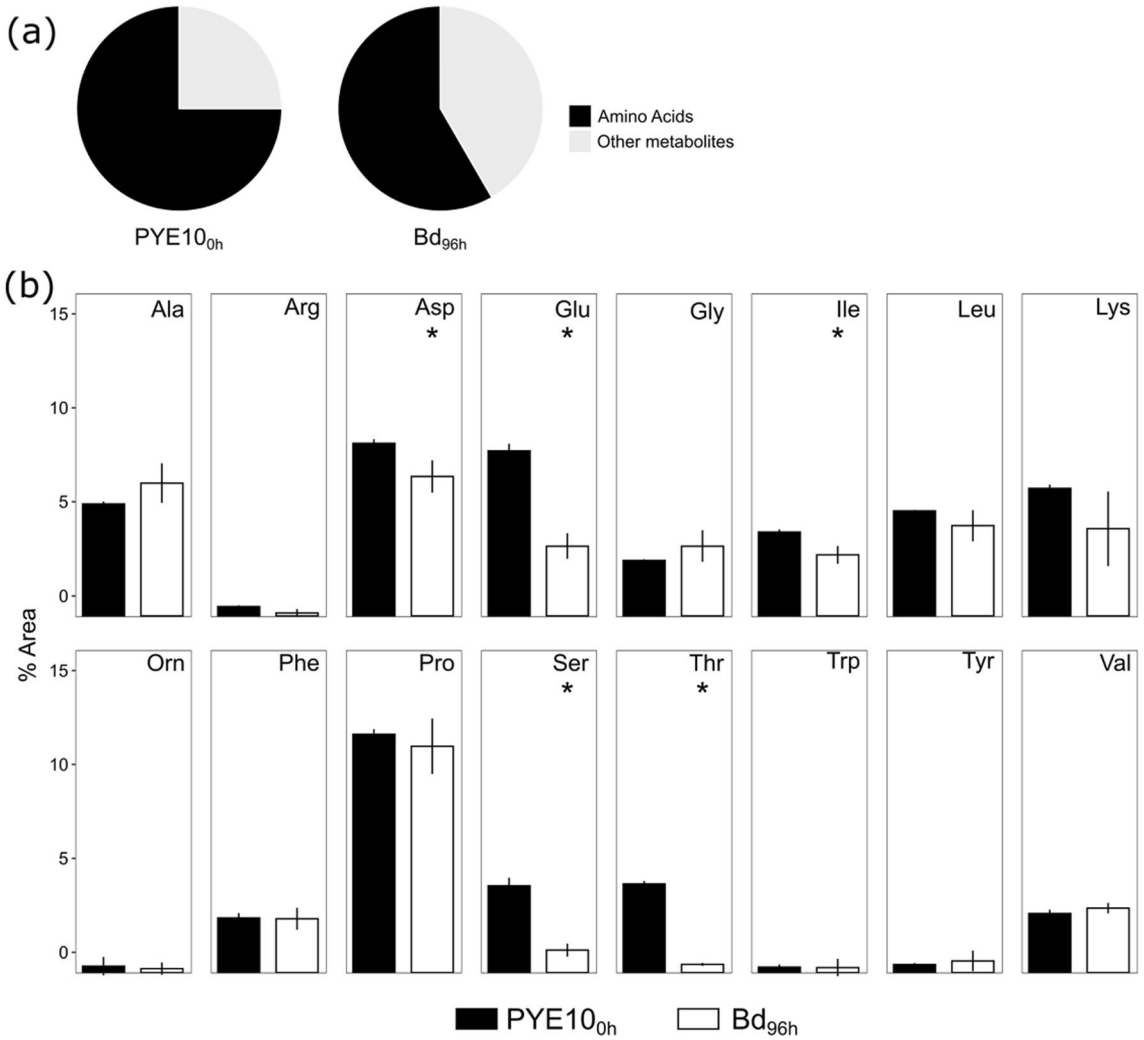

Fig. 4 Relative composition of PYE10 medium during *Bdellovibrio* axenic incubation. **a)** Percentage of amino acid and other metabolites of PYE10 medium at the beginning of the experiment and after 96 h of *Bdellovibrio* incubation. **b)** Amino acid consumption during *Bdellovibrio* axenic incubation (Bd_{96h}) compared with control culture ($PYE10_{0h}$). Error bars indicate the standard deviation of three biological replicates. Ala, alanine; Arg, arginine; Asp, aspartic acid; Glu, glutamate; Gly, glycine; Ile, isoleucine; Leu, leucine; Lys, lysine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine. Asterisks indicate statistically significant differences compared with time $PYE10_{0h}$ (Unpaired *t*-test $pval < 0.02$)

(Zimmerman and Trach 1991). This abundance makes the cytoplasm an exceptionally nutrient-rich environment that facilitates the growth of *B. bacteriovorus* (Vendeville et al. 2011; Zhang 2011). Most of the cytoplasmic proteins are crowded into the vicinity of the cellular envelope, and prey cellular deformation after the bdelloplast formation likely concentrates and increases accessibility to the protein layer (Baquero et al. 2023). *Bdellovibrio* proteases can permeate the altered envelope and use these proteins as substrates to obtain small peptides and amino acids. Based on its genome analysis, *Bdellovibrio* exhibits a wide array of systems dedicated to the uptake of amino acids in the form of di- or tri-peptides (Barabote et al. 2007).

Our research validated the functional metabolism of *B. bacteriovorus* incubated under axenic conditions predicted by metabolic modelling, specifically using the nutrient-rich medium PYE10, in which the primary ingredients are amino acids. The results of our study confirmed the activation of DNA replication (genome number), ATP generation, and biomass production by *Bdellovibrio* under these conditions (Fig. 2). Furthermore, intracellular ATP levels increased by 5-fold when the *Bdellovibrio* cells were incubated in PYE10. The intracellular ATP levels, normalized by biomass, resulted in a 2.5-fold increase, in line with the results obtained in previous studies (Im et al 2018) when incubating *B. bacteriovorus* strain HD100 cells in NB medium. These differences could be attributed to the different nutrient

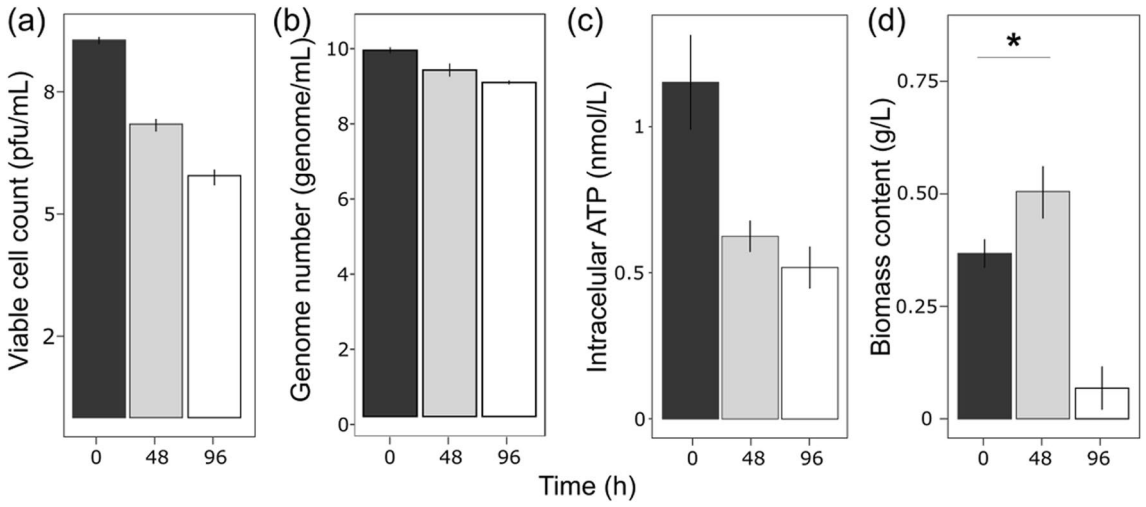

Fig. 5 Growth parameters of *B. bacteriovorus* 109J growing in CAV medium. **a)** Viable cell number of *Bdellovibrio* measured as pfu/mL. **b)** Genome number measured using the *bd2400* housekeeping gene. **c)** Measurement of intracellular ATP levels of *Bdellovibrio* cells. **d)** Total biomass content. Error bars indicate the standard deviation of three biological replicates. Asterisks indicate statistically significant differences compared to time 0 h (Krustal-Wallis t test < 0.03)

concentrations in the PYE10 and NB medium since PYE10 provides a much richer, which could allow higher biomass and intracellular ATP concentration values. The increase in these parameters is likely sustained by amino acid consumption, as confirmed by GC-QTOF-MS analysis (Fig. 4).

The predator, *Bdellovibrio*, is commonly described as host-dependent, which poses a challenge to its efficient application and control. Our findings reveal that *Bdellovibrio* can be cultured as host-independent bacteria while maintaining its effective killing efficiency (Fig. 3). Even after prolonged starvation conditions without prey, predator cells can reduce the prey population as efficiently as in the *Bdellovibrio* control culture during the attack phase. This remarkable result highlights the role of *Bdellovibrio* as a regulator of bacterial populations, not only in aquatic and soil environments (likely invading protein-rich bacterial aggregates or biofilms attached to microbiotic particles (Baquero et al. 2022)) but also in the human commensal microbiota (Iebba et al. 2013; de Dios Caballero et al. 2017). The predator's survival, therefore, does not solely rely on individual predation events.

Furthermore, our experiments pave the way for the rational design of a culture medium to retain the viability of *B. bacteriovorus* cells and facilitate its use in both medical and biotechnological applications. As demonstrated by Im et al. (2018), the incubation of *B. bacterivorus* cells in NB medium or *S. aureus* biofilms resulted in an increased predatory capability and higher intracellular ATP levels as a consequence of amino acid consumption. In this work, we have designed the CAV medium, essentially composed of amino acids that sustain an active metabolism in *B. bacteriovorus*, leading to an increase in biomass (Fig. 5). Amino acids are precursors of biomass components that fuel the bacterial metabolic network at various points (Wang et al. 2019). For instance, glutamate that enters from α-ketoglutarate plays a key role in anaplerotic reactions. Notably, glutamate dehydrogenase directly fuels the tricarboxylic acid cycle by producing α-ketoglutarate. This reaction serves as an excellent source for reducing equivalents, which are essential metabolites for anabolic reactions. Serine, which enters from 3-phosphoglycerate, supplies the upper path of glycolysis. Leucine and isoleucine enter from pyruvate, connecting with the fatty acid synthesis. This study experimentally validated and confirmed the functional capabilities of the *Bdellovibrio* proteolytic machinery and the key role of amino acids in sustaining efficient growth, with or without prey.

In summary, the axenic cultivation of *B. bacteriovorus* under controlled conditions and the modulation of medium composition (PYE10 or CAV) preserves an efficient predator metabolism. Although extensive studies are needed before predator biotechnological and clinical applications become available, the findings presented here open new avenues of research aimed at understanding the nutritional requirements of *B. bacteriovorus*.

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Author contribution CH and MAP conceived the presented idea. CH, VRB, and NHH carried out experiments, data curation, formal analysis, and validation. CH and SS performed the modeling analysis. CH and

MAP supervised the project. CH, VRB, NHH, JN, MAP, RdC, and FB wrote, reviewed, and edited the manuscript. CH, VRB, SS, RdC, FB, JN, and MAP authors discussed and contributed to the final version of the manuscript. CH, JN, and MAP obtained funds for the project.

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Data availability All data generated or analysed during this study are included in the Supplementary Dataset 1 and 2.

Declarations This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

References

Ames J, MacLeod G (1985) Volatile components of a Yeast Extract Composition. J Food Sci 50:9–10

Baquero F, Coque TM, Guerra-Pinto N, Galán JC, Jiménez-Lalana D, Tamames J, Pedrós-Alió C (2022) The influence of coalescent microbiotic particles from water and soil on the evolution and spread of antimicrobial resistance. Front Environ Sci 10:1–13. https://doi.org/10.3389/fenvs.2022.824963

Baquero F, Martínez J-L, Sánchez A, Fernández-de-Bobadilla MD, San-Millán A, Rodríguez-Beltrán J (2023) Bacterial subcellular architecture, structural epistasis, and antibiotic resistance. Biology (basel) 12:640. https://doi.org/10.3390/biology12050640

Barabote RD, Rendulic S, Schuster SC, Saier MH (2007) Comprehensive analysis of transport proteins encoded within the genome of *Bdellovibrio bacteriovorus*. Genomics 90:424–446. https://doi.org/10.1016/j.ygeno.2007.06.002

Barel G, Jurkevitch E (2001) Analysis of phenotypic diversity among host-independent mutants of *Bdellovibrio bacteriovorus* 109J. Arch Microbiol 176:211–216. https://doi.org/10.1007/s002030100312

Capeness MJ, Lambert C, Lovering AL, Till R, Uchida K, Chaudhuri R, Alderwick LJ, Lee DJ, Swarbreck D, Liddell S, Aizawa S-I, Sockett RE (2013) Activity of *bdellovibrio* hit locus proteins, Bd0108 and Bd0109, links type IVa pilus extrusion/retraction status to prey-independent growth signalling. https://doi.org/10.1371/journal.pone.0079759

Chang CY, Hobley L, Till R, Capeness M, Kanna M, Burtt W, Jagtap P, Aizawa SI, Elizabeth Sockett R (2011) The *Bdellovibrio bacteriovorus* twin-arginine transport system has roles in predatory and prey-independent growth. Microbiology (n y) 157:3079–3093. https://doi.org/10.1099/mic.0.052449-0

Chang KL, Ho PC (2014) Gas chromatography time-of-flight mass spectrometry (GC-TOF-MS)-based metabolomics for comparison of caffeinated and decaffeinated coffee and its implications for Alzheimer's disease. PLoS One 9. https://doi.org/10.1371/journal.pone.0104621

Chubukov V, Gerosa L, Kochanowski K, Sauer U (2014) Coordination of microbial metabolism. Nat Rev Microbiol 12:327–340

Cotter TW, Thomashow MF (1992) Identification of a *Bdellovibrio bacteriovorus* genetic locus, hit, associated with the host-independent phenotype. J Bacteriol 174:6018–6024

de Dios Caballero J, Vida R, Cobo M, Máiz L, Suárez L, Galeano J, Baquero F, Cantón R, del Campo R (2017) Individual patterns of complexity in cystic fibrosis lung microbiota, including predator bacteria, over a 1-year period. Mbio 8:e00959-17. https://doi.org/10.1128/mBio.00959-17

Dori-Bachash M, Dassa B, Pietrokovski S, Jurkevitch E (2008) Proteome-based comparative analyses of growth stages reveal new cell cycle-dependent functions in the predatory bacterium *Bdellovibrio bacteriovorus*. Appl Environ Microbiol 74:7152–7162. https://doi.org/10.1128/AEM.01736-08

Dwidar M, Im H, Seo JK, Mitchell RJ (2017) Attack-phase *Bdellovibrio bacteriovorus* responses to extracellular nutrients are analogous to those seen during late intraperiplasmic growth. Microb Ecol. https://doi.org/10.1007/s00248-017-1003-1

Evans KJ, Lambert C, Sockett RE (2007) Predation by *Bdellovibrio bacteriovorus* HD100 requires type IV pili. J Bacteriol 189:4850–4859. https://doi.org/10.1128/JB.01942-06

Gadkari D, Stolp H (1975) Energy metabolism of *Bdellovibrio bacteriovorus*. Arch Microbiol 102:179–185. https://doi.org/10.1007/BF00428366

Gadkari D, Stolp H (1976) Energy metabolism of *Bdellovibrio bacteriovorus** II. P/O Ratio and ATP Pool Turnover Rate. Arch Microbiol 108:125–132

Herencias C, Salgado-Briegas S, Prieto MA (2020a) Emerging horizons for industrial applications of predatory bacteria. in: the ecology of predation at the microscale. Springer International Publishing, Cham

Heirendt L, Arreckx S, Pfau T, Mendoza SN, Richelle A, Heinken A, Haraldsdóttir HS, Wachowiak J, Keating SM, Vlasov V, Magnusdóttir S, Ng CY, Preciat G, Žagare A, Chan SHJ, Aurich MK, Clancy CM, Modamio J, Sauls JT, Noronha A, Bordbar A, Cousins B-J, El Assal DC, Valcarcel LV, Apaolaza I, Ghaderi S, Ahookhosh M, Ben GM, Kostromins A, Sompairac N, Le HM, Ma D, Sun Y, Wang L, Yurkovich JT, Oliveira MAP, Vuong PT, El Assal LP, Kuperstein I, Zinovyev A, Hinton HS, Bryant WA, Artacho FJA, Planes FJ, Stalidzans E, Maass A, Vempala S, Hucka M, Saunders MA, Maranas CD, Lewis NE, Sauter T, Palsson BØ, Thiele I, Fleming RMT (2018) Creation and analysis of biochemical constraint-based models: the COBRA Toolbox v3.0. Nat Protoc 2:1290–1307. https://doi.org/10.1038/nprot.2007.99

Herencias C, Prieto MA, Martínez V (2017) Determination of the predatory capability of *Bdellovibrio bacteriovorus* HD100. Bio Protoc 7:2–10. https://doi.org/10.21769/BioProtoc.2177

Herencias C, Salgado-Briegas S, Prieto MA, Nogales J (2020b) Providing new insights on the biphasic lifestyle of the predatory bacterium *Bdellovibrio bacteriovorus* through genome-scale metabolic modeling. PLoS Comput Biol 16:e1007646. https://doi.org/10.1371/journal.pcbi.1007646

Hespell RB, Odelson DA (1978) Metabolism of RNA-ribose by *Bdellovibrio bacteriovorus* during intraperiplasmic growth on *Escherichia coli*. J Bacteriol 136:936–946

Hespell RB, Rosson R, a, Thomashow MF, Rittenberg SC, (1973) Respiration of *Bdellovibrio bacteriovorus* strain 109J and its energy substrates for intraperiplasmic growth. J Bacteriol 113:1280–1288

Hespell RB, Thomashow MF, Rittenberg SC (1974) Changes in cell composition and viability of *Bdellovibrio bacteriovorus* during starvation. Arch Microbiol 97:313–327

Hobley L, Lerner TR, Williams LE, Lambert C, Till R, Milner DS, Basford SM, Capeness MJ, Fenton AK, Atterbury RJ, Harris MATS, Sockett RE (2012) Genome analysis of a simultaneously predatory and prey-independent, novel *Bdellovibrio bacteriovorus* from the River Tiber, supports in silico predictions of both ancient and recent lateral gene transfer from diverse bacteria. BMC Genomics 13:670. https://doi.org/10.1186/1471-2164-13-670

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. https://doi.org/10.1371/journal.pone.0061608

Im H, Dwidar M, Mitchell RJ (2018) *Bdellovibrio bacteriovorus* HD100, a predator of Gram-negative bacteria, benefits energetically from *Staphylococcus aureus* biofilms without predation. ISME J 12:2090–2095. https://doi.org/10.1038/s41396-018-0154-5

Kaplan M, Chang Y-W, Oikonomou CM, Nicolas WJ, Jewett AI, Kreida S, Dutka P, Rettberg LA, Maggi S, Jensen GJ (2023) *Bdellovibrio* predation cycle characterized at nanometre-scale resolution with cryo-electron tomography. Nat Microbiol 8:1267–1279. https://doi.org/10.1038/s41564-023-01401-2

Lai TF, Ford RM, Huwiler SG (2023) Advances in cellular and molecular predatory biology of Bdellovibrio *bacteriovorus* six decades after discovery. Front Microbiol 14

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Martínez V, Jurkevitch E, García JL, Prieto MA (2013) Reward for *Bdellovibrio bacteriovorus* for preying on a polyhydroxyalkanoate producer. Environ Microbiol. https://doi.org/10.1111/1462-2920.12047

Monk J, Nogales J (2014) Palsson BO (2014) Optimizing genome-scale network reconstructions. Nat Biotechnol 32:447–452. https://doi.org/10.1038/nbt.2870

Morehouse KA, Hobley L, Capeness M, Sockett RE (2011) Three motAB stator gene products in *Bdellovibrio bacteriovorus* contribute to motility of a single flagellum during predatory and prey-independent growth. J Bacteriol 193:932–943. https://doi.org/10.1128/JB.00941-10

Nelson DR, Rittenberg SC (1981) Partial characterization of lipid A of intraperiplasmically grown Partial *Bdellovibrio bacteriovorus*. J Bacteriol 147:869–874

Neto FC, Pilon AC, Selegato DM, Freire RT, Gu H, Raftery D, Lopes NP, Castro-Gamboa I (2016) Dereplication of natural products using gc-tof mass spectrometry: improved metabolite identification by spectral deconvolution ratio analysis. Front Mol Biosci 3:1–13. https://doi.org/10.3389/fmolb.2016.00059

Ogundero A, Vignola M, Connelly S, Sloan WT (2022) Validating flow cytometry as a method for quantifying *Bdellovibrio* predatory bacteria and its prey for microbial ecology. Microbiol Spectr 10(1):e01033-e1121. https://doi.org/10.1128/spectrum.01033-21

Rendulic S, Jagtap P, Rosinus A, Eppinger M, Baar C, Lanz C, Keller H, Lambert C, Evans KJ, Goesmann A, Meyer F, Sockett RE, Schuster SC (2004) A predator unmasked: life cycle of *Bdellovibrio bacteriovorus* from a genomic perspective. Science 303:689–692. https://doi.org/10.1126/science.1093027

Roschanski N, Klages S, Reinhardt R, Linscheid M, Strauch E (2011) Identification of genes essential for prey-independent growth of *Bdellovibrio bacteriovorus* HD100. J Bacteriol 193:1745–1756. https://doi.org/10.1128/JB.01343-10

Rosson R, a, Rittenberg SC, (1981) Pyrimidine metabolism of *Bdellovibrio bacteriovorus* grown intraperiplasmically and axenically. J Bacteriol 146:108–116

Rosson RA, Rittenberg SC (1979) Regulated breakdown of *Escherichia coli* deoxyribonucleic acid during intraperiplasmic growth of *Bdellovibrio bacteriovorus* 109J. J Bacteriol 140(2):620–633

Saralegui C, Herencias C, Halperin AV, de Dios-Caballero J, Pérez-Viso B, Salgado S, Lanza VF, Cantón R, Baquero F, Prieto MA, del Campo R (2022) Strain-specific predation of *Bdellovibrio bacteriovorus* on *Pseudomonas aeruginosa* with a higher range for cystic fibrosis than for bacteremia isolates. Sci Rep 12:10523. https://doi.org/10.1038/s41598-022-14378-5

Sathyamoorthy R, Kushmaro Y, Rotem O, Matan O, Kadouri DE, Huppert A, Jurkevitch E (2021) To hunt or to rest: prey depletion induces a novel starvation survival strategy in bacterial predators. ISME J 15:109–123. https://doi.org/10.1038/s41396-020-00764-2

Seidler RJ, Starr M (1969) Isolation and characterization of host-independent Bdellovibrios. J Bacteriol 100:769–785

Sezonov G, Joseleau-Petit D, D'Ari R (2007) *Escherichia coli* physiology in Luria-Bertani broth. J Bacteriol 189:8746–8749. https://doi.org/10.1128/JB.01368-07

Scheubert K, Hufsky F, Petras D, Wang M, Nothias LF, Dührkop K, Bandeira N, Dorrestein PC, Böcker S (2017) Significance estimation for large scale metabolomics annotations by spectral matching. Nat Commun 8. https://doi.org/10.1038/s41467-017-01318-5

Sockett RE (2009) Predatory lifestyle of *Bdellovibrio bacteriovorus*. Annu Rev Microbiol 63:523–539. https://doi.org/10.1146/annurev.micro.091208.073346

Van Essche M, Sliepen I, Loozen G, Van Eldere J, Quirynen M, Davidov Y, Jurkevitch E, Boon N, Teughels W (2009) Development and performance of a quantitative PCR for the enumeration of Bdellovibrionaceae. Environ Microbiol Rep 1:228–233. https://doi.org/10.1111/j.1758-2229.2009.00034.x

Vendeville A, Lariviere D, Fourmentin E (2011) An inventory of the bacterial macromolecular components and their spatial organization `. FEMS Microbiol Rev 35:395–414. https://doi.org/10.1111/j.1574-6976.2010.00254.x

Wang X, Xia K, Yang X, Tang C (2019) Growth strategy of microbes on mixed carbon sources. Nat Commun 10. https://doi.org/10.1038/s41467-019-09261-3

Zhang Y-HP (2011) Substrate channeling and enzyme complexes for biotechnological applications. Biotechnol Adv 29:715–725. https://doi.org/10.1016/j.biotechadv.2011.05.020

Zimmerman SB, Trach SO (1991) Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*. J Mol Bid 222:599–620