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REVIEW

Insights into *Bdellovibrio* spp. mechanisms of action and potential applications

Monique Waso[1] · Brandon Reyneke[2] · Benjamin Havenga[2] · Sehaam Khan[1] · Wesaal Khan[2]

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Abstract

Recent studies investigating *Bdellovibrio* spp. have found that although this predator predominantly preys on Gram-negative organisms, under certain conditions (nutrient/prey limitation), it will adapt to survive and grow axenically (without prey) or in the presence of Gram-positive bacterial prey. These advances in the understanding of predatory bacteria have stimulated a renewed interest in these organisms and the potential applications of *Bdellovibrio* spp. to the benefit of society. Early studies primarily focused on the application of predatory bacteria as "live antibiotics" in the medical field, probiotics in aquaculture and veterinary medicine and their use in agriculture. Additionally, studies have investigated their prevalence in wastewater and environmental sources. However, comprehending that *Bdellovibrio* spp. may also prey on and target Gram-positive organisms, implies that these predators could specifically be applied for the bioremediation or removal of mixed bacterial communities. Recent studies have also indicated that *Bdellovibrio* spp. may be useful in controlling food spoilage organisms and subsequently decrease our reliance on food additives. This review will thus highlight recent developments in understanding *Bdellovibrio* spp. predation strategies and focus on potential new applications of these organisms for water treatment, food preservation, enhancement of industrial processes, and in combination therapies with bacteriophages and/or antibiotics to combat multi-drug resistant organisms.

Keywords *Bdellovibrio bacteriovorus* · Predation · *Bdellovibrio* applications · Biocontrol

Introduction

Bdellovibrio bacteriovorus (*B. bacteriovorus*) is a well-studied member of the *Bdellovibrio*-and-like-organisms (BALOs), a group of Gram-negative bacteria that multiply by typically predating other Gram-negative bacteria (Sockett 2009). The predator predominantly employs a periplasmic predation strategy; however, using field emission scanning electron microscopy (FESEM), Iebba et al. (2014) proved that *B. bacteriovorus* can prey on Gram-positive *Staphylococcus aureus* (*S. aureus*) in an epibiotic manner. Additionally, Waso et al. (2019) used ethidium monoazide bromide quantitative polymerase chain reaction (EMA-qPCR) to indicate that gene copies from viable *B. bacteriovorus* increased in co-culture with *S. aureus* and *Enterococcus faecium* (*E. faecium*), while the gene copies of the prey cells correspondingly decreased.

Regardless of the predation strategy employed, *B. bacteriovorus* has been found to target and kill pathogenic and opportunistic bacteria belonging to the genera *Pseudomonas*, *Acinetobacter* and *Klebsiella*, amongst many others (Dashiff et al. 2011). Moreover, it has been shown that *B. bacteriovorus* can prey on antibiotic resistant strains of these bacteria (Dashiff et al. 2011; Dharani et al. 2018). Many studies have thus recommended that, much like bacteriophages, BALOs could be used as therapeutic agents to combat multidrug-resistant infections, for crop protection or as environmental (water) clean-up agents (Sockett 2009). In a clinical setting, *B. bacteriovorus* may be particularly useful in topical applications for the treatment of infections of the eyes and burn wounds and could also be used to treat persistent lung infections in cystic fibrosis patients and periodontal infections, amongst other applications (Shanks et al. 2013; Iebba et al. 2014; Bonfiglio et al. 2020). Furthermore,

✉ Wesaal Khan
wesaal@sun.ac.za

[1] Faculty of Health Sciences, University of Johannesburg, PO Box 17011, Doornfontein 2028, South Africa

[2] Department of Microbiology, Faculty of Science, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa

in veterinary medicine, *B. bacteriovorus* has been shown to target *Moraxella bovis*, which causes infection of the cornea and conjunctiva in cattle and can prevent *Salmonella enterica* infection in chicks (Atterbury et al. 2011). Additionally, the probiotic application of *B. bacteriovorus* in aquaculture, for sturgeon and zebrafish, has been investigated, with positive results reported (Chu and Zhu 2010; Cao et al. 2012). In terms of agriculture, the use of *B. bacteriovorus* has been shown to prevent bacterial soft rot in potatoes (Youdkes et al. 2020) and control bacterial blight caused by *Pseudomonas glycinea* (Scherff 1972). It is therefore apparent that *B. bacteriovorus* displays potential to target bacteria in a wide range of industries; however, further research into their predatory strategies should be conducted in order to fully exploit the application of these bacteria as biological control agents (Sockett 2009).

Currently, *Bdellovibrio* research focuses on elucidating the molecular mechanisms which govern their predation activity, while studies aimed at fully characterising their prey range are also being conducted (including Gram-positive bacteria) (Iebba et al. 2014; Rotem et al. 2015). Furthermore, with the use of genome sequencing, research groups are elucidating the metabolic products produced by these predatory bacteria and are investigating the potential biotechnological applications of *Bdellovibrio* (Bratanis et al. 2020). The current research focus is thus no longer solely on the therapeutic use of *B. bacteriovorus* to target problematic Gram-negative pathogens in a clinical setting, but rather to investigate the applications of the varied metabolites and enzymes produced by *B. bacteriovorus* (Bratanis et al. 2020). This review will thus focus on the advances made in understanding *Bdellovibrio* spp. and their employed predation strategies as well as potential new applications for *B. bacteriovorus* in various industries. These will include employing *B. bacteriovorus* as a pre-treatment to primary water treatment systems to improve potable water purification efficiency; combination therapies using *B. bacteriovorus*, bacteriophages and conventional antibiotics; applying these predators as preservatives in food products; employing the metabolic products produced by *B. bacteriovorus* in biotechnological processes or as antimicrobial compounds; and, improving industrial processes such as biofuel production.

Life cycle and predation strategies of *Bdellovibrio* spp.

Bdellovibrio bacteriovorus predominantly employs a periplasmic predation strategy to invade, digest and lyse their target prey; however, in the presence of Gram-positive prey they may employ an epibiotic predation strategy (Lambert et al. 2006; Iebba et al. 2014). The periplasmic predation strategy consists of two phases: the attack phase (AP) where free-swimming *B. bacteriovorus* hunt for prey cells; and the growth phase (GP) where *B. bacteriovorus* grows filamentously within the periplasmic space of an invaded prey cell (within the bdelloplast) (Lambert et al. 2006). A schematic representation and known gene expression profile of *B. bacteriovorus* during (A) host-independent growth and the (B) attack phase, (C) early-growth/attachment phase and (D) growth phase of the periplasmic predation strategy is outlined in Fig. 1.

During the AP, *B. bacteriovorus* cells are small, vibroid to rod-shaped and move at high speeds using a single sheathed flagellum (Sockett and Lambert 2004). While earlier research indicated that prey location by *B. bacteriovorus* predominantly occurs through random collisions (predation may be directly dependent on predator and prey cell density), studies conducted on AP cells indicated that *B. bacteriovorus* exhibits weak chemotaxis towards respirable substrates, specific amino acids and other organic compounds (Strauch et al. 2006). Additionally, Lambert et al. (2003) found that *B. bacteriovorus* 109 J possesses a methyl-accepting chemotaxis protein, while Strauch et al. (2006) found 20 of these proteins and chemotactic machinery (signal environmental changes to the flagellar motor), in *B. bacteriovorus* HD100. It has thus been hypothesised that *B. bacteriovorus* can use weak chemotaxis to locate areas where a higher density of prey cells may be present. In contrast, quorum sensing is essential for the coordinated attack employed by myxobacteria; however, limited evidence on quorum sensing mechanisms in *B. bacteriovorus* has been found (Sester et al. 2020). In fact, Dwidar et al. (2020) demonstrated that acyl-homoserine lactones had no significant effect on *B. bacteriovorus* predation rates, while diffusible signalling factor decreased *B. bacteriovorus* motility by 50% and delayed bdelloplast lysis, thereby decreasing the predation rate and efficiency. Interestingly, Wang et al. (2011) reported that the genome of the epibiotic predator *Micavibrio aeruginosavorus* (*M. aeruginosavorus*) encodes for several quorum-sensing genes; however, the mechanism of how quorum sensing relates to the *M. aeruginosavorus* predation strategy requires further research.

Subsequently, once *B. bacteriovorus* has invaded a prey cell, a growth structure known as the bdelloplast is formed (Fig. 1d). In the bdelloplast, the predator produces an array of enzymes (including proteases, peptidases, glycanases, DNases, RNases and lipases) (Rendulic et al. 2004; Bratanis et al. 2020). These enzymes digest the invaded prey cell from the inside, releasing amino acids, nucleic acids and sugar monomers, which are then absorbed by the predator and utilised to produce progeny (Sockett 2009). Upon maturation of the progeny cells, the prey cell is lysed and the progeny hunt for new prey cells which they can attack, invade and eventually lyse (Lambert et al. 2006). The released predatory cells are dormant and are unable

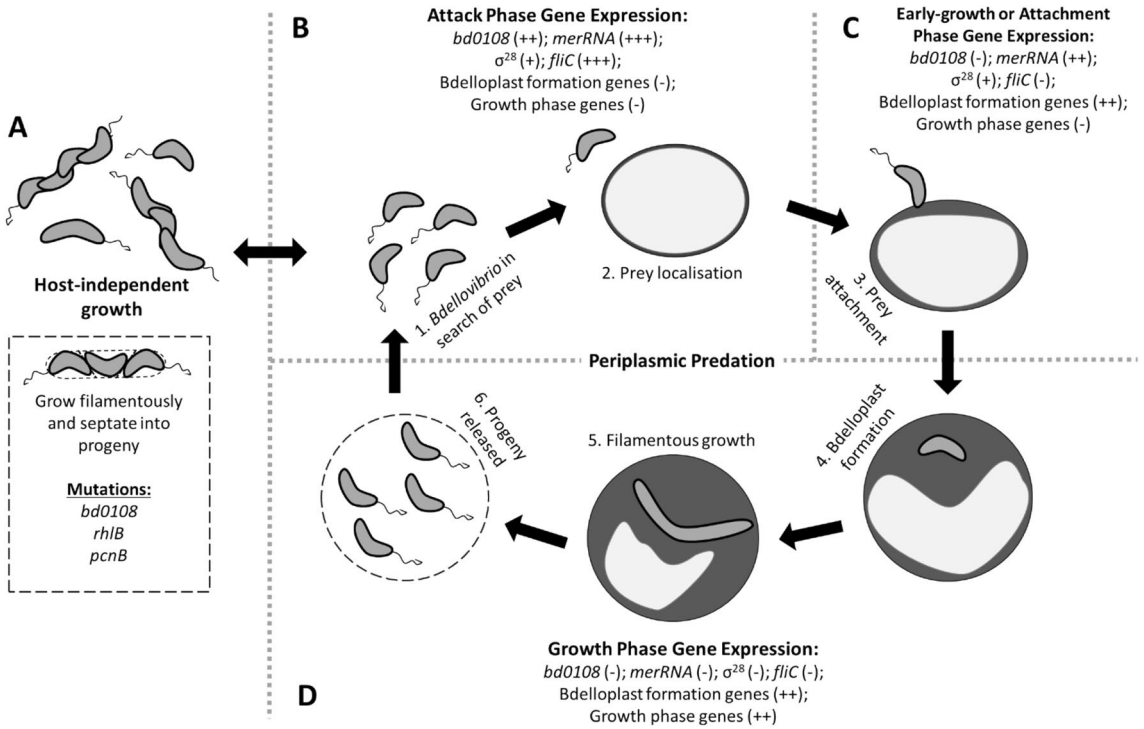

Fig. 1 Schematic representation and primary known gene expression profile of *B. bacteriovorus* during **a** host-independent growth and the **b** attack phase, **c** early-growth/attachment phase and **d** growth phase of the periplasmic predation strategy [Adapted from Rotem et al. (2015)]

to replicate without a host/prey cell (Sockett 2009). In contrast, epibiotic predation entails the binding of the *B. bacteriovorus* to the outside of the prey cell where the predator then proceeds to leach the nutrients from the prey and subsequently grow and produce progeny via binary fission (Deeg et al. 2020).

Some *B. bacteriovorus* strains can also grow axenically (in the absence of prey cells) and have been referred to as host-independent mutants (Fig. 1a). These mutants have lost the ability to predate prey cells. Hobley et al. (2012) found that proteins with the sequence "GGDEF" (which synthesise the small signalling molecule cyclic di-GMP) control the switch between predatory and axenic growth of *B. bacteriovorus*. The authors found that if certain GGDEF proteins are removed, *B. bacteriovorus* can grow on environmental nutrients (axenic growth), while the removal of other GGDEF proteins result in mutant strains which can only survive via predation. The authors highlighted that if the "correct" mutants can be created, which only predate pathogenic bacteria and are unable to survive on extracellular nutrients found in the environment or in the human body (such as nutrients in blood), self-limiting treatments could be developed (Hobley et al. 2012). It is therefore imperative that the molecular mechanisms involved in the predation strategies employed by *B. bacteriovorus* are elucidated in order to fully harness the benefits of using these predators as therapeutic or biocontrol agents.

Insights into the genetic mechanisms that govern *Bdellovibrio* predation strategies

Using gene expression analysis, oligonucleotide microarrays and reverse transcription qPCR, Lambert et al. (2010) reported that approximately 40% of the *B. bacteriovorus* genome encoded for genes potentially involved in the predatory life cycle of the bacterium. Karunker et al. (2013) subsequently used Illumina high-throughput sequencing to further characterise the AP and GP genes of *B. bacteriovorus* in co-culture with *Escherichia coli* (*E. coli*). The authors confirmed that 67% of the genes expressed by *B. bacteriovorus* were active during the GP (1557 genes), 15% were active during the AP (353 genes) and 18% of the genes were simultaneously expressed during the GP and AP. For the genes expressed during the AP, 59% encoded for uncharacterised hypothetical proteins and the remaining 41% were involved in chemotaxis, motility and cell surface composition. The GP genes mostly encoded for ribosome biogenesis, cell division, DNA polymerase, chromosome partitioning proteins and energy metabolism. Furthermore, Rotem et al. (2015) investigated genes upregulated during the AP, attachment or early GP (transitionary phase) and GP of *B. bacteriovorus* and published a model on the potential regulatory pathways involved in the predation activity of *B. bacteriovorus* (Fig. 1).

Specifically, during the AP of the *B. bacteriovorus* cell cycle, non-replicating cells scavenge the environment for suitable prey at a high-speed using a single sheathed flagellum. Flagella encoding genes are thus highly expressed during the AP (Lambert et al. 2006). Similarly, the *bd0108* gene, which is hypothesised to control the extrusion/retraction of a type IVa pilus, is upregulated during the AP (Rotem et al. 2015). These pili are associated with host cell adherence and invasion, twitching motility and fruiting body formation in bacteria such as *Pseudomonas* and *Myxococcus* and exhibit a considerable contractile force. The type IVa pili may thus be important for prey cell adherence (attachment) and invasion in *B. bacteriovorus*. Rotem et al. (2015) also theorised that the first predatory cue is related to the predator sensing an intact bacterial prey envelope via the type IVa pili, which results in the repression of *bd0108* and alters the extrusion/retraction state of the type IVa pilus as CdgA interacts with the pilus regulatory protein complex (Fig. 1c).

Once *B. bacteriovorus* irreversibly binds to a prey cell, its gene expression profile shifts from an AP to a recognition programme. *Bdellovibrio bacteriovorus* then creates a pore in the cell wall of the prey cell to enter the periplasmic space (attachment or early GP) and secretes peptidoglycan-modifying enzymes to seal the pore and form the bdelloplast (Lambert et al. 2010; Lerner et al. 2012; Rotem et al. 2015). The peptidoglycan-modifying enzymes include D-ala-D-ala carboxypeptidases, lytic murein transglycosylase, putative peptidoglycan binding protein and a polysaccharide de-acetylase, which allow the predator to modify the peptidoglycan of the prey cell during the formation of the bdelloplast to facilitate growth (produce progeny using available nutrients) and lyse the prey cell once the progeny have matured (Lambert et al. 2010; Lerner et al. 2012).

Once the second predatory cue (hypothesised to be related to the cytosol of the invaded prey cell) is received and the late signalling pathway is activated, *B. bacteriovorus* enters the GP and actively proliferates within an invaded prey cell (Fig. 1d) (Rotem et al. 2015). During this phase, the σ^{28} regulon, *merRNA* and bdelloplast-formation genes are silenced, while the core GP genes are upregulated (Fig. 1d). Lambert et al. (2010) found that several sensor-regulator genes (transcriptional regulators, two-component sensor-kinases and a CarD-like transcriptional regulator) are upregulated during the GP of *B. bacteriovorus*. These genes are presumably involved in sensing the conditions in the bdelloplast, the secretion of hydrolytic enzymes, *Bdellovibrio* septation and prey cell lysis. Thus, during predation/growth, genes encoding for 173 hypothetical proteins, 6 transcriptional regulators, nucleic acid synthesis, chaperones, peptidoglycan metabolising enzymes, hydrolytic enzymes (protease, esterases, helicases and endonucleases), TonB-like proteins, ABC transporters, ATP synthase and Fe–S clusters, are upregulated.

It is important to note that *B. bacteriovorus* has been found to predominantly prey on Gram-negative organisms (Dashiff et al. 2011), and as such the life cycle and genes involved in the predation activity of *B. bacteriovorus* have mainly been studied in the presence of the model Gram-negative bacterium, *E. coli*. However, Iebba et al. (2014) and Pantanella et al. (2018) showed that *B. bacteriovorus* is able to prey on *S. aureus*. To expand on these studies, Waso et al. (2020a) employed reverse transcription qPCR (RT-qPCR) to investigate the gene expression of an environmental *B. bacteriovorus* isolate in the presence of Gram-negative [*E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*)] and Gram-positive (*E. faecium*) prey. In the presence of all the prey, the AP genes (*bd0108*, *merRNA* and *fliC1*) mostly followed the expected expression pattern. The GP genes (*bd0816* and *groES1*) were then induced in the presence of the Gram-negative prey, however, in the presence of the Gram-positive prey, the expression of these genes remained low. The authors subsequently hypothesised that the second predatory cue (prompts predator growth) may be lacking in the presence of Gram-positive bacteria or that *B. bacteriovorus* requires more time to produce the hydrolytic enzymes required to degrade Gram-positive prey.

Recently, Deeg et al. (2020) reported on the isolation of *Bdellovibrio qaytius* (*B. qaytius*) which preferentially preyed on *Paraburkholderia fungorum* in an epibiotic manner. While the genome of *B. qaytius* encoded for a hydrolytic enzyme arsenal similar to the epibiotic predators *M. aeruginosavorus* and *Bdellovibrio exovorus*, it also encoded a complex genomic complement similar to periplasmic predators (significant overlap between the genomes of *B. qaytius* and *B. bacteriovorus*) (Deeg et al. 2020). It was found that *B. qaytius* represents a basal branch within the *Bdellovibrio* genus, which may indicate that epibiotic predation is an ancestral predation strategy and that *Bdellovibrio* spp. have subsequently evolved to use periplasmic predation to prey on a wider range of bacteria (epibiotic predators generally have a narrower prey range). Additionally, Deeg et al. (2020) hypothesised that epibiotic predation may be more readily employed by *B. bacteriovorus* in natural environments, but that this predation strategy may not always be observed by laboratory strains of *B. bacteriovorus*.

Applications of *Bdellovibrio* spp.

The application of next-generation sequencing technologies and real-time PCR assays have advanced the understanding of the molecular mechanisms of *B. bacteriovorus*, with subsequent novel applications investigated. For example, the genome of *B. bacteriovorus* HD100

(type strain) was found to encode for a vast range of specialised hydrolytic enzymes which facilitate their predatory life cycle (Rendulic et al. 2004; Lerner et al. 2012). An example of these unique enzymes is evolutionarily diversified class C penicillin-binding-proteins (PBPs) (D,D-endopeptidases), which aid the predator in creating a pore in the prey cell wall in order to enter the periplasmic space and to change the shape of the invaded prey cell to a rounded bdelloplast (Lerner et al. 2012). *Bdellovibrio bacteriovorus* also produces a unique lysozyme (DslA), which acts on deacetylated peptidoglycan (found in invaded prey bacteria) and facilitates the lysis of a prey cell when nutrients have been depleted and progeny have been produced (Harding et al. 2020). However, only three potential loci for the production of secondary metabolites have been identified: a siderophore (aerobactin; thought to aid in iron acquisition during growth within a prey cell); an aromatic polyketide (phenolic lipid and alkylpyrone synthase with unknown function); and a carotenoid (protection against oxidative stress during prey cell invasion) (Sester et al. 2020). Thus based on the vast number of hydrolytic enzymes that are produced by this bacterium, the potential of the *B. bacteriovorus* secretome to serve as a novel source of lytic proteins for use in the medical field or in the food, agriculture and biotechnology industries, will predominantly be focused on in this review (Fig. 2b–d) (Bratanis et al. 2020).

Bdellovibrio metabolites as alternative antimicrobials and biotechnological tools

Based on the bioinformatics analysis of the genome of *B. bacteriovorus* HD100, an estimated 293 potential lytic proteins including 150 proteases and peptidases, 10 glycanases, 20 DNases, 9 RNases and 15 lipases have been identified (Rendulic et al. 2004). The arsenal of hydrolytic enzymes produced by *Bdellovibrio* spp. have thus been identified as an unexplored source of potentially valuable products that could be exploited in various biotechnological processes (Fig. 2d) (Rendulic et al. 2004; Bratanis et al. 2020).

Monnappa et al. (2014) then demonstrated the efficacy of extracellular enzymes produced by a host-independent *B. bacteriovorus* strain against biofilms formed by the Gram-positive bacterium *S. aureus.* The authors established *S. aureus* biofilms in 96-well plates and added the supernatant of a host-independent culture of *B. bacteriovorus* to the wells. Using scanning electron and fluorescent microscopy, results indicated that the preformed biofilms were significantly disrupted (> 75%) following a 24-h exposure time period. The enzymes present in the supernatant were subsequently identified (using liquid chromatography with tandem mass spectrometry, quantitative protease assays, and qualitative and quantitative DNase activity assays) as proteases (serine proteases Bd1962 and Bd2269 and carboxypeptidase Bd0306) and DNases. Furthermore, the authors reported that exposure of the *S. aureus* cells to the supernatant of the host-independent *B. bacteriovorus* culture reduced the ability of

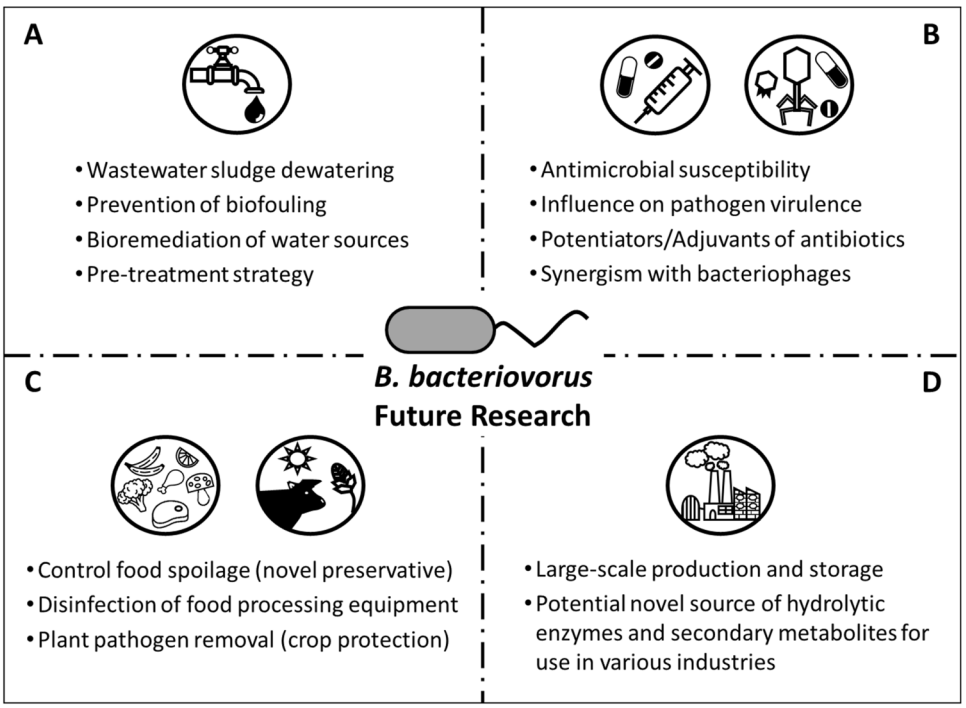

Fig. 2 Research needs and potential application of *B. bacteriovorous* in the **a** water, **b** medical, **c** food and agriculture and **d** biotechnology industries

the prey cells to infect human epithelial cells by fivefold. It was thus hypothesised that the proteases and DNAses produced by *B. bacteriovorus* may reduce the virulence of certain pathogens such as *S. aureus*, thereby highlighting the potential of *Bdellovibrio* derived enzymes to serve as antimicrobials (Fig. 2b).

Hydrolytic enzymes produced by *Bdellovibrio* may also be employed in the biotechnology industry as antibody-modulating tools (Bratanis et al. 2020). Various commercially available bacterial-derived hydrolytic enzymes are implemented by pharmaceutical companies for the analysis and quality control of monoclonal antibodies as part of the research and development of biological therapeutic agents. These enzymes function by increasing the sensitivity of mass spectrometry, by fragmenting or digesting proteins into smaller peptides, prior to analysis (Bratanis et al. 2020). For example, *Bdellovibrio* serine protease K (BspK) and *Bdellovibrio* elastase-like serine protease (BspE) display promise for use in the biotechnological industry, as they have been shown to hydrolyse immunoglobulin G and immunoglobulin A, respectively, and may thus contribute to the analysis of these immunoglobulins (Bratanis et al. 2020).

Moreover, it has recently been highlighted that *Bdellovibrio* spp. have the potential to improve certain industrial processes by acting as a biocontrol agent of contaminating bacteria or by limiting the use of detergents and solvents (Bratanis et al. 2020). For example, microalgae-derived biofuels have been identified as an environmentally friendly energy solution; however, ponds wherein microalgae are cultivated are prone to bacterial contamination. The bacterial contaminants not only directly compete with the microalgae for available nutrients but may also kill the microalgae (Li et al. 2018). Li et al. (2018) subsequently aimed to isolate a predatory bacterium that could be used for the biocontrol of bacterial contaminants and to promote the growth of the microalgae, *Chlorella* USTB-01. During the study, the authors isolated *Bdellovibrio* USTB-06 and reported that the addition of the predatory bacterium to the *Chlorella* culture, reduced the concentration of contaminating bacteria by 5 logs, while the growth of *Chlorella* increased by 37% (as compared to the control cultures).

In comparison, *B. bacteriovorus* has been investigated for the recovery of intracellular bio-products produced by Gram-negative bacteria, by serving as a lytic agent (Bratanis et al. 2020). For example, biopolymers produced by Gram-negative and Gram-positive bacteria, are attractive alternatives to the use of petroleum-based plastics (Martínez et al. 2016). However, due to their intracellular storage, conventional recovery methods (i.e. mechanical disruption, centrifugation/filtration, enzymatic digestion, use of solvents/detergents) are complicated, time-consuming, costly and may be detrimental to the environment (Bratanis et al. 2020). To overcome these disadvantages, Martínez et al. (2016) reported on the use of *B. bacteriovorus* HD100 as a downstream lytic agent for the recovery of polyhydroxyalkanoate (PHA), an accumulated intracellular bacterial polyester, as the predator is able to lyse Gram-negative producer strains and release the PHA into the extracellular environment. Results from the study indicated that *B. bacteriovorus* HD100 allowed for the recovery of 54–60% of the accumulated intracellular PHA, while the use of a $PhaZ_{Bd}$ (PHA depolymerase) knockout mutant *B. bacteriovorus* HD100, improved accumulated PHA recovery to 80%. The authors thus concluded that *Bdellovibrio* displays potential to be used as an inexpensive lytic biological agent for the recovery of intracellular bio-products from Gram-negative organisms (Martínez et al. 2016).

Preservative in the food industry

Foodborne and plant pathogenic bacteria are significant threats to food-security and sustainable agriculture, as they cause losses in crop yield and affect produce safety. Additionally, the lack of appropriate bactericidal chemicals, that are safe to use on food products and regulations limiting the use of antibiotics for pathogen control in the food industry, have impeded the effective control of foodborne and plant pathogenic bacteria (Olanya and Lakshman 2015). This is a significant concern as many plant pathogenic microbes such as *Erwinia*, *Pectobacterium* and *Pseudomonas* spp. also predispose fruits and vegetables to colonisation by enteric pathogens such as *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* (Olanya and Lakshman 2015). The development of safe, alternative bactericidal treatment strategies, for the control of foodborne pathogens and the prevention of food spoilage, is thus a priority research area.

As *B. bacteriovorus* has been described as a common member of the human intestinal microbiota, with an inability to grow in eukaryotic cells (Raghunathan et al. 2019), it has the potential to be used as an environmentally friendly preservative in the food industry (Fig. 2c) (Ottaviani et al. 2020). While investigating the ability of *B. bacteriovorus* to serve as a biological control agent of meat products, Ottaviani et al. (2020) monitored the potential of *B. bacteriovorus* 109 J to control the growth of *E. coli* on chicken slices and in canned beef. Results from the study indicated that *B. bacteriovorus* 109 J was able to reduce *E. coli* growth on the chicken slices and in canned beef by 4.3 log and 2.1 log, respectively, after 6 h. Additionally, *B. bacteriovorus* 109 J displayed activity against all the tested *E. coli* strains, including toxigenic and multidrug-resistant strains. The use of *B. bacteriovorus* in meat-based foods may thus minimise the use of conventional additives in the food industry and prolong the shelf-life of meat products. In comparison, Saxon et al. (2014) investigated the potential of

B. bacteriovorus HD100 to prevent the formation of brown-blotch lesions on the surface of *Agaricus bisporus* supermarket mushrooms. Results from the study indicated that *B. bacteriovorus* HD100 was successfully able to target and decrease the concentration of the mushroom spoilage bacterium, *Pseudomonas tolaasii*, and reduce blotch severity on the treated mushrooms. However, the authors also noted that in some cases, the *B. bacteriovorus* pre-treatment resulted in the overgrowth of *Enterobacter* spp., which were resistant to predation, on the mushrooms. The study therefore not only highlighted the ability of *B. bacteriovorus* to potentially prevent food spoilage in post-harvest storage situations, but also highlighted the importance of assessing the influence of *B. bacteriovorus* treatment in different scenarios, as *Enterobacter* spp. may be pathogenic.

Potable water pre-treatment strategy

Globally, water scarcity and access to safe water is a major concern and the development of cost-effective water treatment systems is a priority, especially for regions where access to piped water is limited or unavailable. While the antifouling potential and possible application of *B. bacteriovorus* as a biolysis agent for wastewater treatment has been investigated (Yilmaz et al. 2014; Özkan et al. 2018), the potential application of this predator as a biocontrol or bioremediation agent for potable water treatment will be focused on in this section of the review (Fig. 2a).

Kim et al. (2013) applied *B. bacteriovorus* HD100 as an environmentally friendly pre-treatment to filtration to decrease the microbial load in feed water. The water samples were seeded with *E. coli* and subsequently pre-treated with *B. bacteriovorus* HD100 for 48 h, whereafter the samples were subjected to membrane filtration. The *B. bacteriovorus* then significantly reduced the bacterial load (by > 4 logs) in the samples prior to membrane filtration, which subsequently limited the formation of biofilms on the membrane filters. Additionally, the pre-treatment decreased flux decline and resistance across the membrane, which validated the finding that pre-treatment with the predatory bacteria decreased membrane fouling (confirmed with microscopy). The application of *B. bacteriovorus* as a pre-treatment in potable water treatment plants may thus extend the life span of membrane filters in this industry.

Waso et al. (2020b) then applied *B. bacteriovorus* as a pre-treatment to solar disinfection and solar photocatalysis for the purification of harvested rainwater. Briefly, synthetic rainwater was seeded with *E. faecium* and *K. pneumoniae* and the samples were pre-treated with an environmental *B. bacteriovorus* isolate for 72 h. The authors found that *B. bacteriovorus* effectively enhanced the disinfection of the Gram-negative *K. pneumoniae* using solar disinfection and solar photocatalysis as the cell counts were reduced by 9.30 logs in both treatments. However, for the Gram-positive *E. faecium*, higher cell count log reductions (8.00 logs) were observed in the samples which were only subjected to the solar-based treatments (i.e. not pre-treated with the predatory bacteria).

While it is evident that *B. bacteriovorus* could be applied to improve water treatment processes (specifically to eradicate Gram-negative bacteria), upscaling the production of high concentration inocula of *B. bacteriovorus*, to treat larger volumes of water, could prove challenging. Future studies will thus need to investigate bioreactor systems and optimal growth conditions to upscale the production of *Bdellovibrio*, as well as strategies for the effective removal of the prey cells (filtration systems) from the *Bdellovibrio* inocula (Fig. 2a, d). Cao et al. (2019) also noted that liquid *Bdellovibrio* preparations have a short shelf-life, with significant losses in *Bdellovibrio* cell viability, if the preparations are stored for extended time periods. The authors suggested that it may thus be beneficial to spray dry and encapsulate *Bdellovibrio* preparations prior to application and subsequently demonstrated that the encapsulated *Bdellovibrio* powder remained viable for up to 120 days at room temperature. It was also safe for shrimp farming and exhibited significant antibacterial effects, at a concentration of 0.8 mg/L, against shrimp-pathogenic vibrios. It may thus be feasible to develop encapsulated *Bdellovibrio* preparations to treat other water sources however, the optimal dose will need to be investigated.

Bdellovibrio combination treatment strategies

The increased occurrence of antimicrobial resistant bacteria, yeast and fungi in various industries (e.g. medical, food, water), has highlighted the need for alternative treatment strategies to effectively combat these microorganisms (Bratanis et al. 2020; Pérez et al. 2020). The application of *Bdellovibrio* spp. in combination with bacteriophages or antibiotics or the application of *Bdellovibrio* enzymes as antimicrobials, have subsequently been proposed as alternative treatment strategies (Bratanis et al. 2020; Pérez et al. 2020).

Lytic bacteriophages are bacterial viruses which infect and lyse host bacterial cells (Wu et al. 2017). However, while bacteriophages have been applied to target bacterial pathogens in various industries, their narrow host range and the potential for the development of bacteriophage resistance by the target organism, limits their widespread application (Wu et al. 2017). The combination of bacteriophages with other treatment strategies may, however, overcome these limitations and improve overall treatment efficiency (Fig. 2b).

For example, Hobley et al. (2020) recently reported on the dual predation of *E. coli* by a rosette-tailed-like bacteriophage (*Siphoviridae* family) and *B. bacteriovorus* HD100, with the combination predation resulting in the eradication (< 10 CFU/mL) of the *E. coli* in liquid culture. The authors concluded that the experimental data may be explained by assuming three different prey phenotypes, i.e. sensitive to both predators, resistant to bacteriophage only or resistant to *B. bacteriovorus* only. Thus, while each predator reduced *E. coli* availability, both predators were able to coexist and exert different selective pressures on the *E. coli* population to effectively control the bacterial numbers.

Similarly, as *Bdellovibrio* spp. are inherently resistant to β-lactam antibiotics and antifolates, it has been suggested that *Bdellovibrio* spp. could serve as potentiators or adjuvants to conventional antibiotics (such as penicillins, carbapenems and trimethoprim), thereby reducing the development of antibiotic resistance and increasing the effectiveness of antibiotics against multidrug-resistant pathogens (Bratanis et al. 2020; Marine et al. 2020). Im et al. (2017) investigated the efficiency of co-therapy involving *B. bacteriovorus* HD100 with violacein (bisindole Gram-positive specific antibiotic) against six different bacterial species [*S. aureus*, *Bacillus cereus* (*B. cereus*), *Staphylococcus epidermis* (*S. epidermis*), *E. coli*, *K. pneumoniae* and *Acinetobacter baumannii* (*A. baumannii*)] in mono-, co- or polymicrobial cultures. Overall, the *B. bacteriovorus* and violacein co-therapy reduced the combined viable cell counts of *S. aureus* and *A. baumannii* by > 99.8%. The application of the co-therapy against a polymicrobial community (*S. aureus*, *A. baumannii*, *B. cereus* and *K. pneumoniae*) also resulted in a 99.96% reduction in the total pathogen count. It was thus hypothesised that *Bdellovibrio* and violacein exhibited a synergistic effect, resulting in increased antimicrobial activity.

Although the efficiency of co-therapy has been demonstrated, it is important to assess which antibiotics *B. bacteriovorus* is resistant to, and could be combined with, for therapeutic use (Fig. 2b). However, it is difficult to assess *B. bacteriovorus*' sensitivity to antibiotics, as the predator cannot be evaluated in standard broth microdilution or minimum inhibitory concentration assays, as it does not produce colonies on agar plates (Cho et al. 2019). This limitation in culturing *B. bacteriovorus* has however, recently been addressed by Marine et al. (2020) through the use of a liquid-based assay, which entails the measurement of *B. bacteriovorus* growth in co-culture with a standardised concentration of stationary phase prey cells (*E. coli*) in a nutrient-limited medium and the presence/absence of different antibiotic concentrations. The viability of *B. bacteriovorus* can then be determined using double-layer agar overlays, while the concentration of *E. coli* can be estimated using the optical density of the cultures after 24 h of incubation. Of the 21 conventional antibiotics tested, trimethoprim exhibited the lowest antimicrobial effect against *B. bacteriovorus* and could potentially be combined in co-therapy with this predator. Additionally, the sensitivity of *B. bacteriovorus* to antibiotics targeting membrane integrity could be assessed through the use of viability dyes with molecular assays. For example, Waso et al. (2019) used the EMA viability dye in combination with qPCR as a molecular-based method to monitor the concentration (increase or decrease) of viable *B. bacteriovorus* in co-culture with Gram-negative and Gram-positive bacteria. These methodologies may thus allow for the accurate selection of antibiotics for use in combination with *Bdellovibrio* and increase the spectrum of co-therapy options.

Conclusions and future research

Studies investigating predation mechanisms and the subsequent effect of these predation strategies on prey populations thus enhance our understanding of how and where *Bdellovibrio* can be applied to benefit humans, animals and the environment. *Bdellovibrio* and their metabolic products also have great value in the medical field as therapeutic agents for the treatment of skin, lung and eye infections. Additionally, they could be used to enhance available therapeutics that may have become ineffective or as alternative therapeutic options to treat multidrug-resistant infections. This may be achieved by combining live *Bdellovibrio* inocula or their antimicrobial metabolites, with conventional antibiotics or bacteriophages.

Bdellovibrio spp. are a rich source of potential biotechnological agents (based on their enzymatic arsenal) which could be used to enhance industrial processes or in the pharmaceutical industry for antibody analysis via mass spectrometry. They also have value as biocontrol/bioremediation agents that could be applied specifically for potable water treatment and in the food industry as food preservatives. Future studies should thus explore the vast range of metabolic products produced by these predators, as they could serve as alternative antimicrobials (enzyme based antimicrobial compounds) and could be employed for various biotechnological purposes [e.g. improved protein analysis, enhancement of industrial processes (bioplastic recovery, antibacterial agents)]. The development and optimisation of large-scale predatory bacteria inocula is also crucial for application in biocontrol or bioremediation strategies and industrial fermentations.

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Declarations

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