

BDELLOVIBRIO BACTERIOVORUS: MORE THAN JUST A BACTERIAL HUNTER

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Abstract: *Bdellovibrio bacteriovorus* is a predatory gram-negative delta-proteobacteria that preys on other gram-negative bacterial species. With the surge in the cases of Anti-Microbial Resistance and the demand to replace conventional antibiotics, this predatory attribute of *B. bacteriovorus* has led to a vast number of studies in the last decade alone. These studies demonstrated various aspects of predation and proposed using *B. bacteriovorus* as an alternative new approach to conventional antibiotics in humans. Here we review the current progress made in this promising new area of research to develop novel antibacterial approaches.

1. Introduction. 2. Characterizing *B. bacteriovorus*. 2.1. Requirements for survival. 2.2. Predation overview. 2.3. Attack phase. 2.4. Prey entry and invasion. 2.5. Growth phase. 2.6. Survival of attack phase cells. 2.7. Synergistic effect of *B. bacteriovorus* and conventional antibacterials. 3. *B. bacteriovorus*: a possible “live antibacterial”. 3.1. *In-vitro* evidence. 3.2. *In-vivo* evidence. 3.3. Obstacles. 4. Industrial applications of *B. bacteriovorus*. 5. Summary

Keywords: antimicrobial, *Bdellovibrio bacteriovorus*, gram-negative, live antibiotic, predatory species

1. Introduction

Bdellovibrio bacteriovorus belongs to a large family of tiny vibrioid-shaped predatory organisms known as *Bdellovibrio*-and-like-organisms (BALO). BALO species possess a single flagellum and an average size of 0.2–0.5 $\mu\text{m} \times 1.2\text{--}1.5 \mu\text{m}$. These species are divided into two major categories of α - and δ -proteobacteria. Predation is the only way for BALOs (including *B. bacteriovorus*) to reproduce and they choose gram-negative bacteria as their prey for replication [3, 36, 48, 74]. Specifically, *B. bacteriovorus* is a gram-negative obligate aerobe and a highly motile δ -proteobacteria (size: approximately 1 $\mu\text{m} \times 0.2 \mu\text{m}$). Its ability to feast on microbes makes it ubiquitous and leads to its presence ranging from salt and freshwater to the human gut [3].

Since its discovery in 1962 *Bdellovibrio bacteriovorus* has not been studied extensively compared to other bacterial species. It may be because a dire need for an alternative to antibiotics did not exist either until the start of the twentieth century. Nevertheless, as the cases of antimicrobial resistance (AMR) started to, more frequently, make headlines in newspapers and scientific journals alike, the scientific community diverted their attention

to a more formidable threat in the form of AMR. To further substantiate the situation, World Health Organization, as a warning, provided an estimate of 10 million deaths globally, by 2050, at the hands of antibiotic resistance [59]. This marked an increase in the number of studies, especially in the last decade, proposing *Bdellovibrio bacteriovorus* as a “live antibiotic” in humans to primarily treat gram-negative infections; however, the first-ever human trials are yet to be conducted.

In this review, we place special emphasis on research from the last decade that supports the potential use of *B. bacteriovorus* as a possible replacement for presently available antibiotics in treating gram-negative bacterial infections (Fig. 1). Next, we will discuss the predatory lifestyle of *B. bacteriovorus* and the critical studies conducted to illuminate its various features (Fig. 2). We then review the efficacy of *B. bacteriovorus* and certain significant obstacles to its use as a “live antibiotic” in humans. Essential findings regarding treatment of gram-negative infections, interaction with the immune system, efficiency in removing bacterial colonies, toxicity towards human cell lines, will be discussed in the proceeding paragraphs. Finally, we will also emphasize the predatory ability of *B. bacteriovorus* by discussing

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Bdellovibrio bacteriovorus: Research from Last Decade

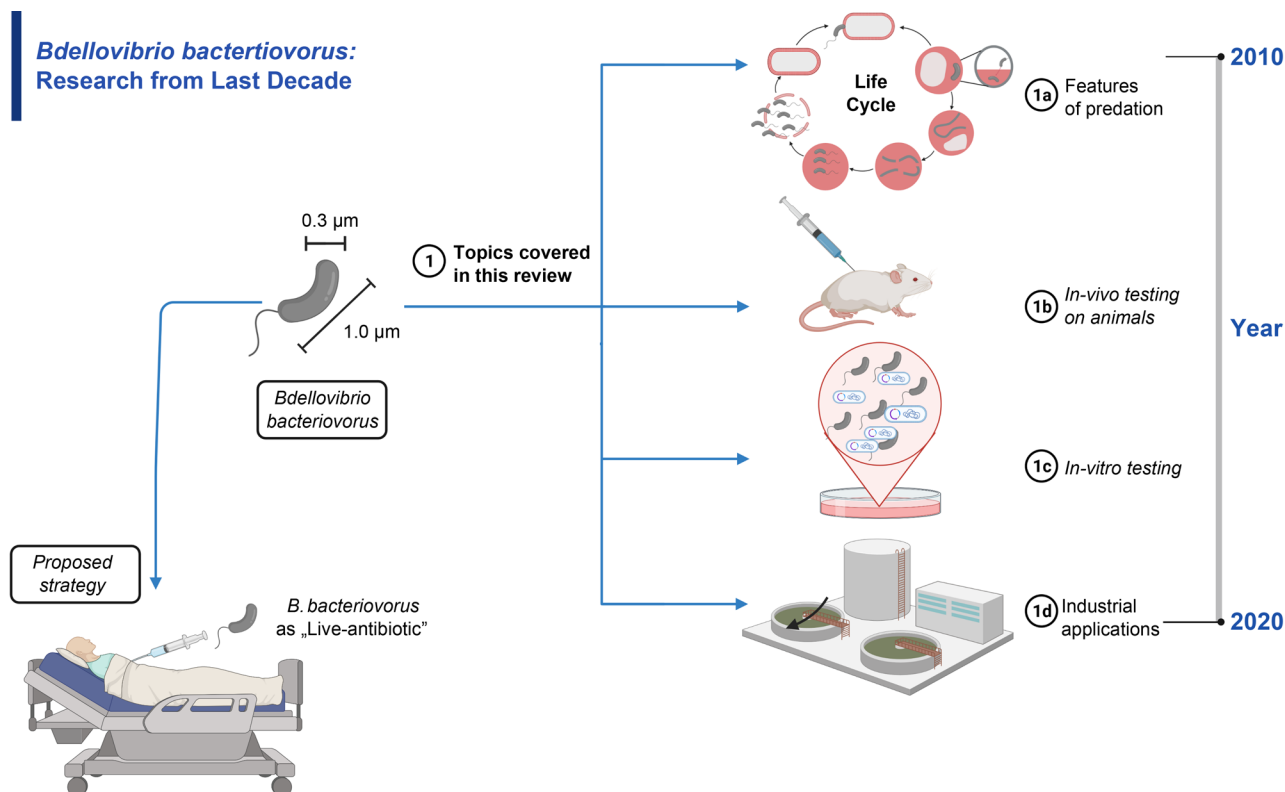


Fig. 1. (Adapted from “The Role of ILC2s in Asthma Pathogenesis”, by BioRender.com (2022). Retrieved from <https://app.biorender.com/biorender-templates>)

Description: A visual representation of the type of research conducted in the last decade concerning *B. bacteriovorus*. The review uses studies from the following areas to establish the antibacterial properties of the predator. 1a) The features of predation highlighted by the studies from the last decade provide new insight into the predation process. Followed by the 1b) *In-vitro* testing of the species to establish *B. bacteriovorus* as non-cytotoxic toward human cell lines and effective in preying on known gram-negative pathogens. Moreover, using this information to conduct 1c) *In-vivo* animal studies to test whether the knowledge gained from *in-vitro* studies translates well in animals. But also, to use a mammalian model in mice to study the predator's safety to gain some idea of its efficacy in humans. Lastly, 1d) Industrial applications provide a different perspective on *B. bacteriovorus*. As its ability to prey on gram-negative species is put to use in various commercial settings. Ranging from its role in the food industry, it can prevent spoilage from gram-negative species like *E. coli*. To a more sophisticated field like Biotechnology, using its hydrolytic enzymes, it can obtain hard-to-extract biopolymers like polyhydroxyalkanoates (PHAs) from the cytoplasm of gram-negative bacteria. Adding to the reputation of *B. bacteriovorus* for being efficacious against gram-negative bacteria and being multifaceted. With most of the reviewed studies suggesting the use of this species as an antibiotic replacement, substantial evidence to merit the safety of this predator as an antimicrobial agent in humans is still missing. There are other areas of *B. bacteriovorus*, including its genome, that have received considerable attention in the last decade but lie beyond the scope of this review.

studies exploring the commercial applications of *B. bacteriovorus* as a possible solution to various industrial dilemmas caused by gram-negative species, such as wastewater treatment, protecting food items by prolonging their shelf-life, and so forth. Thus, the review consolidates the reputation of *B. bacteriovorus* as a valuable addition to the list of proposed replacements for antibiotics. And a good asset in the fight against AMR.

2. Characterizing *B. bacteriovorus*

2.1. Requirements for survival

B. bacteriovorus is an oligotroph that tends to favor nutritionally deficient media for colony formation. Under non-symbiotic conditions *B. bacteriovorus* acts

as a strict aerobe but reports of survival under anoxic conditions also exist once the predator enters the periplasm [62]. In terms of optimal growth conditions, a temperature of 30°C and a pH ranging between 7.5 and 8 helps the predator grow [63]. Furthermore, *B. bacteriovorus* can be separated into host-dependent (HD) and host-independent (HI) strains, although the HI strain can revert to an HD lifestyle if introduced to prey cells [27]. For an HD strain to locate prey cells it must swim (Fig. 2). However, a lack of prey has been proposed to involve the use cyclic-di-GMP (CdG) effectors and two flagellar-brake proteins by the predator to help reactivate the swim-arrest, which affects the predator's swimming pattern and energy metabolism, and the predator survives as a result. *B. bacteriovorus* starts swimming again in search of prey once the prey is re-introduced [60].

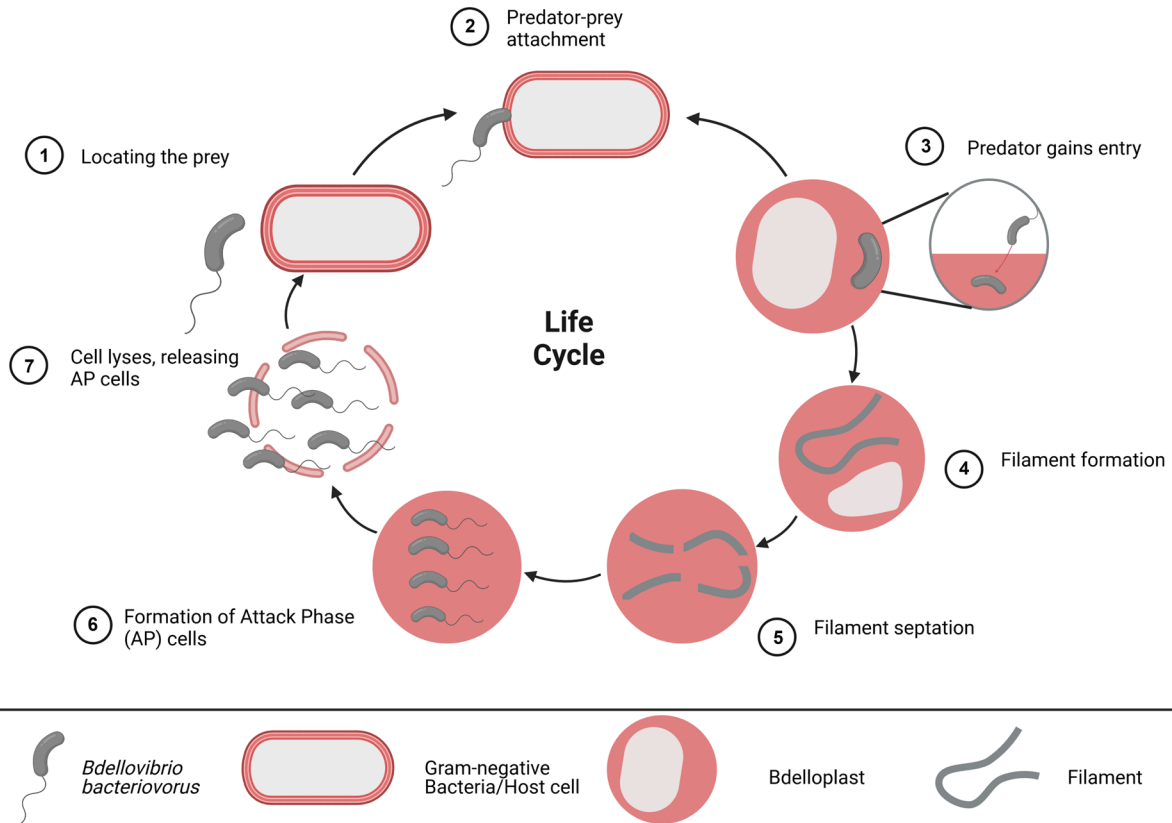


Fig. 2. (Adapted from “Lytic and Lysogenic Cycle”, by BioRender.com (2022). Retrieved from <https://app.biorender.com/biorender-templates>)

Description: The life cycle of the predatory, gram-negative bacterial species *B. bacteriovorus*. The life cycle is divided into an Attack Phase (AP) and a Growth Phase (GP). AP: (1. Predator searches for and locates its prey (a gram-negative bacterial host) using chemotaxis followed by swimming towards the prey using its flagellum. 2. *B. bacteriovorus*, upon finding its prey, attaches to its cell wall). GP: (3. Predator uses its massive arsenal of hydrolytic enzymes to enter the prey cell and feed on the prey cell cytoplasm using hydrolases. The host cell is now called a bdelloplast. 4. The predator grows and forms a polynucleoid filament, almost the size of the prey cell itself. 5. Filament undergoes septation. 6. A new line of AP cells is released. 7. The bdelloplast peptidoglycan undergoes deacetylation by the newly formed AP cells. Thus, they are released. And the cycle repeats itself [14, 23, 36, 40, 53, 63, 67, 68].

2.2. Predation overview

HD strain has four life stages (Fig. 1 represents the complete life cycle of *B. bacteriovorus*) [63]. First stage consists of locating and recognizing the prey cell (a gram-negative species) [68]. Upon locating the prey cell, the predator then invades the cell's periplasm by penetrating its peptidoglycan cell wall [63]. Following invasion, the *B. bacteriovorus*-containing prey turns into a round-shaped cell called a bdelloplast [67], and growth phase initiates. The growth phase strategy of *B. bacteriovorus* relies on periplasmic predation, instead of epibiotic predation [40, 53]. Although, it's been suggested by Deeg et al. that *Bdellovibrio*, as a genus, might be ancestrally epibiotic [14]. During growth, predator utilizes the prey cell contents as a source of nutrition. However, there is evidence of rebuttal by the prey cells in certain cases, in the form of transcriptional products released to repair the damage caused by the predator, especially to the cell wall. *B. bacteriovorus*, regardless of the minor defense put up by the affected organism, fin-

ished off its prey [36]. Once the prey has no more nutrition to offer, *B. bacteriovorus* septates and new progeny is formed. The new attack phase cells then burst out after the prey cell has undergone lysis [23, 63, 67]. The AP cells then search for other prey cells and the aforementioned process repeats itself.

2.3. Attack Phase

During attack phase *B. bacteriovorus* uses its flagellum [35], to propel it towards the prey cell, followed by a reversible attachment of the two [67, 68]. Due to the temporary nature of this attachment the predator also has the option to detach itself from the prey if the prey is considered unsuitable [6, 67]. In case detachment happens, there are reports of this leaving the prey cell wall discontinuous [1]. On the other hand, the process of attachment that is currently agreed-upon is mediated by Type IV pili, present on the pole across the flagellum in the form of tiny retractile filaments made of protein [55]. Furthermore, the involvement of Type IV pili is

reported to be restricted to the predator-prey attachment only [22]. Multiple factors including PilF, Tfp and PilG have been reported to be essential in the assembly of Type IV pili [9]. However, the specific role of pili in *B. bacteriovorus* life cycle relies on a few important elements. These elements include the PilA protein, which has been reported as essential in recognizing the prey [22]. Moreover, a high level of PilA expression has been observed in both the attack and growth phase, which hints at its participation in the early stages of predation [41]. Other examples of assembly proteins for Type IV pili include PilT2, which has been observed to assist in biofilm predation [10].

2.4. Prey entry and invasion

For the predator to enter and invade the prey cell the formation of pore in the Bdelloplast peptidoglycan is deemed the most crucial step. It was initially thought that certain enzymatic reactions were behind this step [67]. Subsequently, glycanases were proposed to be the main component in hydrolysing the bdelloplast peptidoglycan [70]. In terms of bdelloplast formation, both glycanases and peptidases were at one point considered to be heavily involved in the process. However, later in a study by Tudo et al. it was peptidases, instead of glycanases, that were highlighted as the reason for peptidoglycan hydrolysis [71]. On the other hand, in the last decade this area was further explored by Lerner et al. (2012) who reported that a couple of penicillin-binding proteins (PBP) could be the reason for the rounding of the prey cell [38]. These proteins are expressed at an earlier stage of predation and perform the functions of DD-endopeptidase and DD-carboxypeptidase [38]. In addition, the bdelloplast formation might be a strategy used by the two enzymes to ward off any further attacks by other *B. bacteriovorus* cells, while at the same time allowing entry of the attached predator cell through the peptidoglycan layer with its entire structure intact. Furthermore, the authors of the same study did not associate the two enzymes to any important role during prey invasion.

Genomic sequencing of the predator has also revealed some other components that are important for pore formation and predator entry [16, 55]. In one such study by Lambert et al., multiple enzymes were identified to facilitate the entry of *B. bacteriovorus* into the prey cell [37]. These enzymes included deacetylases, proteases and glycanases, specifically, deacetylase weakened the peptidoglycan layer of the prey [37]. This finding disagrees with previous studies where peptidoglycan deacetylation was thought to protect the bdelloplast from premature lysis by glycanase. The same study further considered the initial formation of the pore to be assisted by deacetylation and not hampered by it [37].

2.5. Growth Phase

After prey invasion, the pore in the outer membrane of the prey cell, used by the predator for entry, is sealed. Shortly after, morphological changes are triggered in the prey cell resulting in the formation of a round shaped cell (bdelloplast) [38]. Furthermore, transpeptidases are released by the predator to aid the peptidoglycan layer of the bdelloplast in building intracellular resistance against osmotic pressure [34]. Next, the predator rapidly depletes the nutrient stores of the bdelloplast to aid its replication. Moreover, recent findings have illuminated the role of *B. bacteriovorus* nucleases (Bd0934 and Bd3507) in the digestion of nucleic acids inside the bdelloplast [5]. The predator grows in the form of a filament (69), along with duplication of its chromosomes. Also, the genetic material of the *B. bacteriovorus* HD strain has been observed to only replicate inside the bdelloplast [42]. In the case of *B. bacterivorous*, a delay has been reported between chromosome replication and cell division which causes a filament to form temporarily, similar events have been observed during the replication of *Streptomyces* [42, 58].

In a study by Fenton et al. the final stages of *B. bacteriovorus* replication inside the bdelloplast were observed [23]. The predator cell simultaneously elongates from both poles. After that, once the elongation reaches maximum length the predator cell produces new progeny cells and septation is complete. For septation to initiate it is imperative for the predator to completely deplete the nutrients of the prey cell. Still, the exact mechanisms deployed by the predator to duplicate its chromosomes and divide into new AP cells are unknown. The newly formed AP cells then lyse what remains of the bdelloplast to exit the prey cell. An inverse proportionality has also been reported between the number of new AP cells and the amount of time it takes them to exit the bdelloplast [23]. In the end, the progeny cells use the pores created in the remains of the bdelloplast to exit the prey. Furthermore, Harding et al. suggested the involvement of lysozyme DslA (acts on peptidoglycans that are GlcNAc-deacetylated) to facilitate prey exit [26]. Following their exit, the new *B. bacteriovorus* cells devote a small period to elongate a bit further, and once they are set to initiate a new attack phase the elongation ends [23].

2.6. Survival of attack phase cells

A bacterial predator like *B. bacteriovorus* is not completely protected against extracellular environmental threats. While searching for prey the predator must first deal with its environment which is usually limited in nutrients and contains harmful secondary metabolites secreted by other organisms. These metabolites play

an important role in the ecology and regulation of the extracellular environment, and hence in the interaction between the predator and other bacterial species [72]. No such reports currently exist that highlight specific molecules targeting *B. bacteriovorus*. However, a few observations have been made regarding molecules like cyanide that has been observed to safeguard its source (a bacterial species) from predation [47]. Also, pH and particular carbohydrates have been shown to have a deterrent effect on predation [13].

2.7. Synergistic effect of *B. bacteriovorus* and conventional antibacterials

Under normal conditions, *B. bacteriovorus* shares its habitat and source of nutrients with other organisms. This not only leads to competition amongst different species but also prevents *B. bacteriovorus* from feeding on the entire population of prey [20]. So, to compensate for this type of predator-prey dynamic a possible alternative like combination therapy with other antimicrobials might prove useful. However, prior to pairing the predator with an antibiotic, to increase its preying capacity, it is essential to evaluate the sensitivity of *B. bacteriovorus* towards different antibiotics. With regards to this topic multiple studies have been published in the last decade that expand on this subject. In one such study by Marine et al., the susceptibility of the predator towards several antibiotics was investigated. This was achieved by assaying a liquid co-culture of *E. coli* and *B. bacteriovorus*. The study highlighted Trimethoprim as the antibiotic toward which the predator showed resistance. This was attributed to natural resistance, by the authors, since dihydrofolate reductase of *B. bacteriovorus* displays no affinity [43].

Furthermore, there are reports of a synergistic effect produced by combining *B. bacteriovorus* and Violacein (a bisindole antibiotic that targets gram-positive bacteria) to control the growth of gram-positive species, which otherwise lie outside the spectrum of bacterial classes that *B. bacteriovorus* can negatively impact [18]. Im et al. further tested *B. bacteriovorus* and Violacein against a polymicrobial population comprising of gram-negative *A. baumannii* and *K. pneumoniae*, and gram-positive *S. aureus* and *Bacillus cereus*. Both *B. bacteriovorus* and Violacein, when tested separately, effectively reduced the viability of gram-negative and positive strains, respectively. Surprisingly, when used together, both showed an enhanced efficacy in reducing the viability of their respective strains. Furthermore, the antibacterial activity of *B. bacteriovorus* was not negatively impacted in any way by Violacein and vice versa [30]. These findings encourage consideration of combination therapy involving live antibacterials like *B. bacteriovorus* and conventional antibiotics such as

Violacein to treat polymicrobial infections. Furthermore, this type of combination therapy could reduce prevalence of antibiotic resistance genes by preventing horizontal gene transfer. This is so as using conventional antibiotics concurrently with *B. bacteriovorus* might induce bacterial resistance and the predator provenly digests the genetic material of gram-negative pathogens besides feeding on the rest of the prey cell contents [46].

Lastly, aside from antibiotics, studies from the past decade have also focused on the use of bacteriophages in combination with *B. bacteriovorus* for pathogen removal. Hobley et al. investigated this concept and observed a mutual tolerance between *B. bacteriovorus* and bacteriophages. The same study showed the predator having a supplementary effect on the ability of phages to control gram-negative species like *E. coli* [28]. This example of predator-bacteriophage cooperation is a promising solution to not only phage resistance but also the predator's inability to destroy the entire prey population.

3. *B. bacteriovorus*: a possible "live antibacterial"

3.1. *in-vitro* evidence

In the last decade, *in-vitro* testing has been a recurring theme and has provided vital evidence regarding a few aspects of *B. bacteriovorus*. These aspects can be divided into two main categories of prime interest: toxicity and predatory ability/mechanism. Recently, *in-vitro* studies have tested the cytotoxicity of *B. bacteriovorus* on human cells lines like Human Corneal Limbal Epithelium (HCLE) cells [64], keratinocytes, kidney epithelial cells, liver epithelial cells, and monocytes both from blood and spleen [25]. In these cells, cytotoxicity assays showed that *B. bacteriovorus* was unable to generate any inflammatory response and did not affect the viability of these cells [25]. Monnappa et al. (2016) studied Lung epithelial (NuLi-1) and Intestinal epithelial cells, which can produce cytokines against foreign pathogenic bacteria but showed no signs of cytokine induction by *B. bacteriovorus*. The same study also supported the predator's potential to be considered non-cytotoxic to mammalian cells [45].

In terms of its predatory ability/mechanism, recent studies have also focused on the ability of *B. bacteriovorus* to prey upon/reduce host cell viability of Multi-drug Resistant (MDR) bacteria, including nosocomial pathogens that produce beta-lactamases [12, 33]. For instance, *B. bacteriovorus* can eradicate gram-negative strains that are resistant to previously discontinued antibiotics like colistin [15]. Moreover, *B. bacteriovorus* has shown a protective ability toward human

cells. For example, the predator protects epithelial cells from pathogens like *Pseudomonas* sp. while protecting the viability of the epithelial cells or maintaining the microbial balance in the human gut of pediatric patients preventing Dysbacteriosis-induced diseases like celiac disease, inflammatory bowel disease, and cystic fibrosis [29, 19, 32].

3.2. *in-vivo* evidence

One of the concerns with administering *B. bacteriovorus* as a “live antibiotic” is the possibility of abnormally triggering the immune system. However, multiple studies have reported the non-toxicity of *B. bacteriovorus* in animal models [64–66]. In one such study by Shatzkes et al. a slight increase in specific proinflammatory cytokines was observed in response to *B. bacteriovorus* in mice models, but the level returned to normal after almost 24 hours [65]. One possible explanation behind this lack of sustained immune response in mice against *B. bacteriovorus* is the presence of neutrally-charged-Lipopolysaccharides (LPS) structure of *B. bacteriovorus* as opposed to negatively charged LPS in other gram-negative bacteria [66]. Furthermore, in terms of cytotoxicity in animal models, *B. bacteriovorus* is reported to show no cytotoxicity towards the ocular surface of mice [57]. However, its contribution is insubstantial in clearing the pathogens from the corneal epithelium [56].

B. bacteriovorus has proven to be highly beneficial, even in removing plaque-causing gram-negative pathogens like *Yersinia pestis*, which devastates small animals like mice and can also inflict massive harm on humans by its transmission through flea vectors [24, 59]. Also, there is evidence of *B. bacteriovorus* protecting C57BL/6/SKH-1 mice from *Y. pestis* *in-vivo* [24, 59]; however, the exact mechanism of action is unknown. On the other hand, the predator does not protect Balb/c mice from *Y. pestis* [24]. Furthermore, in terms of deadly human pathogens, *Shigella flexneri*, especially the carbenicillin-and-streptomycin-resistant strain, claims around one million human lives annually [39]. The efficacy of *B. bacteriovorus* has been tested against this strain in animal models like zebrafish larvae. It was reported that *B. bacteriovorus* kills the pathogen without inducing any adverse immune system effects. On the contrary, the activity of the immune system is enhanced when working alongside *B. bacteriovorus*, and towards the end of predation *B. bacteriovorus* is removed by the host immune system [75]. Moreover, Shatzkes et al. observed *B. bacteriovorus* finding it challenging to prey upon species in the vasculature compared to a specific immune-privileged organ due to difficulty in locating the prey and removing the predator itself [66].

3.3. Obstacles

A considerable amount of literature has been published establishing the therapeutic ability of *B. bacteriovorus* [12, 15, 19, 24, 25, 29, 32, 45, 59, 64–66, 75], and proposing its use in *in-vivo* human studies. However, certain findings contradict this concept [2, 21, 17, 31]. In a study by Duncan et al. prey motility was identified as a hurdle to predation by *B. bacteriovorus*. The predator finds it challenging to attach to motile prey like *Vibrio cholerae* compared to non-motile prey due to the drag force that it experiences [17]. Among other obstacles to predation, Baker et al. reported a disparity, in terms of pathogen removal by *B. bacteriovorus*, in the human serum and buffer. The authors investigated the interaction between *B. bacteriovorus* and one of the nosocomial pathogens: Carbapenemase-producing *Klebsiella pneumoniae* [2]. The interaction was tested both in human serum and in the buffer. The predator eradicated *K. pneumoniae* initially but took 19 h longer to attach to the prey in the human serum model compared to the buffer. Upon microscopic observation, contact with the serum changed the shape of the predator from vibroid, which indicates predation, to a round shape. More importantly, after predation, *K. pneumoniae* grew back in buffer and serum, but further research is required to substantiate these findings. The same authors speculated that it was due to plastic resistance, but no further attempts were made to explore the reasons for re-growth [2]. Furthermore, Im et al. reported a similar impact of human serum on *B. bacteriovorus* [31]. The study involved the cells of *K. pneumoniae*, *E. coli*, and *Salmonella enterica* that were subjected to predation in human serum. The osmolality of human serum and serum albumin inhibited predation. As the osmolality of the serum rose the activity of *B. bacteriovorus* declined. Furthermore, the serum albumin caused inhibition by binding and coating the cells of the *B. bacteriovorus* [31]. Finally, predation is hindered by indole, which is a metabolite in many gram-positive and gram-negative bacteria and is released during the stationary phase [21]. Indole reduces the motility of *B. bacteriovorus* during the attack phase, which is integral for the predator to locate its prey and slows down the exit of the predator from inside the bdelloplast [21]. Addressing these hurdles will therefore be essential before performing any *in-vivo* studies on human subjects.

4. Industrial applications of *B. bacteriovorus*

In the last decade, numerous studies have surfaced that investigated the unorthodox use of *B. bacteriovorus* as a biocontrol agent in the food, environmental,

healthcare industries, and its biotechnological applications. This indicates that the use of *B. bacteriovorus* is not solely restricted to public health but probably extends further. In the food industry, the predator has been mainly tested against food spoilage, like its use in controlling the growth of bacterial species of genus *Dickeya* and *Pectobacterium*. These species are pathogenic towards plants, specifically potatoes, as they tend to damage the roots, as a result, the agricultural production suffers [77]. Furthermore, *B. bacteriovorus* displayed activity which was dependent on its concentration, and the presence of glucose was observed to provide protection from predation. Among other studies on prevention of food spoilage by *B. bacteriovorus*, the predator suppresses the growth of *Pseudomonas tolasii*, thereby protecting the post-harvest mushroom (*Agricus bisporus*) from the toxin, Tolaasin, it produces. Thus, the predator reduces the appearance of brown lesions on the mushroom pileus, making it more appealing to buyers [61]. Similarly, other post-harvest food products such as lettuce and carrots have also been shown to be protected from *E. coli* or *Salmonella* using *B. bacteriovorus* [50]. In addition, the shelf life of pre-packaged meat products has been reported to significantly increase when *B. bacteriovorus* is used to mediate the removal of spoilage bacteria like *E. coli* [51]. Finally, as a biocontrol agent, *B. bacteriovorus* (SOIR-1 strain) also provides protection against phytopathogens such as *Xanthomonas campestris* and *Pantoea* sp. strain BCCS responsible for causing rotting of potato tubers and onion bulbs, respectively [49].

As a biocontrol agent *B. bacteriovorus* has been tested in the fishing industry as well. In one such study by Cao et al. the predator was used against shrimp pathogens as a bio-disinfectant [7]. Later, the authors of the same study were, for the first time, able to encapsulate the predator which prolonged its stability and viability at room temperature for approximately 120 days [8]. Furthermore, *B. bacteriovorus* has also shown the ability to alter the feeding behavior of the Mandarin fish: *Siniperca chuatsi*, from live baitfish (costly) to pellet feed (more economically viable), as well as enhance its growth and survival rate. The predator does that by enhancing the expression of genes responsible for regulating appetite: Neuropeptide Y (*Npy*) and Agouti-related peptide (*Agrp*), and by controlling the growth of *Aeromonas* (pathogenic bacteria) in the intestinal microflora of the fish [11]. Among environmental applications of *B. bacteriovorus*, it can be used in pre-treatment of rainwater, as a lead-in to solar disinfection and solar photocatalytic treatment, to control the growth of bacterial pathogens such as *Klebsiella pneumoniae* [73]. Like rainwater treatment, *B. bacteriovorus* has also been used in the Wastewater treatment (WWT) plants as a cleaning agent as it can prey upon

the sludge microorganisms and prevent the membrane from blocking in membrane bioreactors [52].

With regards to the healthcare industry, *B. bacteriovorus* has shown the ability to target oral pathogens. With the oral microbiota being highly diverse the predator has been reported to prey on certain periodontal pathogens [54]. It has been observed that *B. bacteriovorus* is able to prey on aerobic bacterial species but not the anaerobic ones (such as *Fusobacterium nucleatum* and *Porphyromonas gingivitis*) inside the oral cavity. Moreover, it has also been suggested that *B. bacteriovorus* can help control the microbial growth of antibiotic-resistant gram-negative *Enterobacter cloacae* JK-2, in knee wear simulators (used to design improved Total Knee Replacement (TKR) implants) [3].

Lastly, *B. bacteriovorus* also has applications as a biotechnological tool. In a study by Martinez et al. *B. bacteriovorus* was successfully used in extracting the hard-to-extract biopolymers like polyhydroxyalkanoates (PHAs) (found in the cytoplasm of both gram-negative and positive bacteria), using its enormous range of hydrolytic enzymes. The predator produced up to 80% yield without any breakage of the product [44]. Moreover, the same study also proposed the use of *B. bacteriovorus* instead of conventional extraction techniques like high-pressure homogenization, which have high costs and a low yield [44]. Furthermore, there are reports of new biotechnological tools like BspK (a protease K derived from *B. bacteriovorus*) that can help speed up the quality control and evaluation process of new therapeutic antibodies. Usually, the quality control process involves cleaving antibodies to their more minor constituents to facilitate their analysis. However, the currently in-use tools like Trypsin are inefficient and ineffective in producing high quality products. This prolongs the approval process for therapeutic antibodies, thus depriving patients in urgent need of these antibodies. Therefore, proteases like BspK can be a valuable addition to the quality control process [4].

5. Summary

In summary, *B. bacteriovorus* has shown potential as a therapeutic agent across various fields and can be considered a possible antibiotic replacement. However, the substantial evidence to merit the safety of this predator as an antimicrobial agent in humans is still missing. The *in-vitro* studies have given some promising results, but issues like prey motility, when used along with indole and the scientific gaps in the knowledge about the function of each gene in the bacterial genome, pose severe hurdles in the route to develop this species as a replacement to traditional antibiotics. The future research should therefore focus on accumulating

conclusive evidence regarding the safety of this predatory species in humans alongside exploring the genome of *B. bacteriovorus*.

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