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Predatory Bacteria: A Novel Approach to Antibiotic Substitution

Shiney George[1], R. K. Sharma[2], Ritam Hazarika[3], Snigdha Hazarika[4], Rofique Ahmed[5] and Donna Phangchopi[6]

[1]Dept of Microbiology, [4]Dept. of Pharmacology, [6]Dept. of Animal Genetics and Breeding, Lakhimpur College of Veterinary Science Joyhing, Assam Agricultural University, Lakhimpur, Assam (787 051), India

[2]Dept. of Microbiology, [3]Dept. of Animal Biotechnology, [5]Dept. of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science Khanapara, Assam Agricultural University, Assam (781 022), India

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Corresponding shineygeorge0@gmail.com

0000-0003-1540-5900

ABSTRACT

Antibiotics are an effective means of treating infectious bacterial diseases. However, the extensive use of antibiotics in the recent past has resulted in antibiotic-resistant bacteria which interfere with disease treatment and recovery. There is an urgent need for novel medicines to treat Gram-negative infections because there are not enough antibiotics in development to address projected and present demands. One novel approach to antibiotic alternatives is the use of living predatory bacteria which can naturally prey upon other Gram-negative bacteria. These predators are ubiquitous in a wide variety of manmade and natural environments and depending upon their feeding habits are classified as obligatory or facultative predators. All the obligate predators are classified under the umbrella terminology *Bdellovibrio* and like organisms (BALOs). *Bdellovibrio bacteriovorus* is by far the most studied BALOs and have long been recognized as a potential therapeutic, water clean-up, and biocontrol agent and source for discovering novel biotechnological tools for research. Antibiotics won't be replaced by pills containing predatory microorganisms any time soon. However, understanding these bacteria could help us prepare for a time when many medications cannot treat infections that are multi-drug resistant. A paradigm change is necessary since it may appear counterintuitive to cure a bacterial infection by giving another bacterium. The burden of an infection on the host must be taken into account while evaluating the prospective implications of treating a Gram-negative bacterial infection with the administration of a live predatory bacterium.

KEYWORDS: Antibiotics, Predatory bacteria, *Bdellovibrio*, *B. bacteriovorus*, BALOs

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1. INTRODUCTION

In the natural evolutionary competition for survival, nothing guarantees that the human species will survive the ongoing war against pathogenic microorganisms unless we search for newer alternatives. Global health and economic issue are caused by antimicrobial resistance (AMR). There is an urgent need for novel medicines to treat Gram-negative bacterial infections because there are not enough antibiotics in development to address projected and present demands (Atterbury and Tyson, 2021). The UN convened a high-level meeting on antimicrobial resistance in September 2016 to affirm international political commitments to addressing the spread of multi-drug resistance (MDR) bacteria (Shatzkes et al., 2017a). A wide taxonomy of predatory bacteria was identified, including facultative and obligatory predators that are distinguished by their feeding habits. Only the α-proteobacteria (genus *Micavibrio*) and δ-proteobacteria (Families: *Bdellovibrionaceae, Bacteriovoracea, Peredibacteraceae, Halobacteriovoraceae,* and *Pseudobacteriovoraceae*), collectively referred to as BALOs, are required to lead a predatory lifestyle (Paix et al., 2019, Rotem et al., 2014). While facultative predators quickly adopt a saprophytic lifestyle in the absence of suitable food, obligatory predators must consume the cells of their prey in order to exist (Korp et al., 2016). Since the discovery, antibacterial drugs saved millions of lives by combating fatal infectious diseases and enabling physicians to make advances in surgery, organ transplantation, cancer chemotherapy, and the use of artificial devices (Lewis, 2020). When prey is exhausted, the predator is divided by septation into several flagellated progeny cells, followed by host cell lysis and progeny release, after which the cycle begins a new (Lambert et al., 2015, Avidan et al., 2017). Numerous artificial and natural habitats contain predatory bacteria. In addition to sewage, these include soil and other aquatic environments such as rivers, lakes, the open ocean, and wastewater treatment plants (WWTPs) (Oyedara et al., 2016, Paix et al., 2019). Its prey range includes several known human pathogens that either already has acquired or are at significant risk of acquiring resistance to antibiotics, such as enterohemorrhagic *Escherichia coli*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Pseudomonas*, and *Salmonella* (Dwidar et al., 2012, Shatzkes et al., 2016). By discovering and describing BALOs Proteases (BspK and BspE) with documented enzymatic activity on human antibodies, this potential can be demonstrated (Bratanis et al., 2017, Bratanis and Lood, 2019). Saxon et al. (2014) and Ottaviani et al. (2019) demonstrated that *B. bacteriovorus* could control *E. coli* and other spoilage bacteria in meat products. The majority of therapeutic MAbs that are now approved belong to the IgG1 subclass and they are largely utilized to treat autoimmune disorders and various cancers (Irani et al., 2015). The term "biological control," refers to the employment of any organism to eradicate an undesirable population of another, is gaining popularity because of its affordability and little negative impacts on the environment, animals, and the general public's health (Kergunteuil et al., 2016). Additionally, it has been suggested that *B. bacteriovorus* could be helpful for freshwater farming as a biological control agent for the viruses, *V. cholerae* and *V. parahaemolyticus* that affect shrimp (Cao et al., 2015, Kongrueng et al., 2017). Large-scale cultivation of newly introduced or naturally occurring strains could be employed as a broad-spectrum biocontrol agent to fight phytopathogens that would otherwise harm the crops (McNeely et al., 2017, Youdkes et al., 2020). Predatory bacteria thrive in environments with high prey density and naturally occur in WWTPs (Feng et al., 2016, Yu et al., 2017). The BALOs are inherently non-pathogenic to mammals (Gupta et al., 2016, Willis et al., 2016, Shatzkes et al., 2017a).

2. WHY IS AN APPROACH FOR ANTIBIOTIC SUBSTITUTION NEEDED?

The indiscriminate use of antibiotics, among other reasons, has put pressure on bacteria to evolve resistance against these drugs, leading to the emergence of untreatable superbugs thus, drawing a severe public health concern to the global scientific community. Compounding this issue is that the antibiotic discovery void over the last 30 years has resulted in the current shortage of new antibiotics in the pipeline. Also, the gradual rise of animal and human-associated MDR bacterial infection and Lack of viable antibiotics suggests an urgent need for novel antibiotic alternatives.

3. GLOBAL APPROACH

A global action plan on antimicrobial resistance was endorsed at the World Health Assembly in May 2015. This global action plan aims to ensure the prevention and treatment of infectious diseases with safe and effective medicines. The UN convened a high-level meeting on antimicrobial resistance in September 2016 to affirm international political commitments to address the spread of MDR bacteria (Shatzkes et al., 2017a). The lack of viable antibiotics has urged researchers to consider new approaches to overcome the resistant and multi-drug resistant (MDR) bacteria.

4. ANTIBIOTICS ALTERNATIVES

Ideal alternatives to antibiotics should be non-toxic or have no side effects on animals, easily eliminated from the body or consist of short-term residues, be stable in the feed and animal gastrointestinal tract, be easily decomposed,

not induce bacterial resistance, not affect the environment, not affect palatability, not destroy the normal intestinal flora of animals, kill or inhibit the growth of pathogenic bacteria, enhance the body resistance to the disease, improve feed efficiency and promote animal growth, and have good compatibility. Many antibiotic alternatives have been tested and tried *viz.* prebiotics, probiotics, phytobiotics, antimicrobial peptides, anti-biofilm peptides, antibodies, vaccines, bacteriophages etc; however, there are no alternatives that currently meet all the properties of an ideal substitute.

One novel approach to antibiotic alternative is the use of living predatory bacteria, such as *Bdellovibrio bacteriovorus*, tiny Gram-negative bacteria found ubiquitously in soil and aquatic environments that naturally invade and kill other Gram-negative bacteria.

5. PREDATORY BACTERIA

Predatory interactions are ubiquitous. They not only exist between animals but also between microbes. Predatory bacteria use other bacteria or yeasts as a food source. They actively hunt and kill their neighbors to later consume their macromolecules as nutrients. Predatory bacteria are ubiquitous in a wide variety of manmade and natural environments. These include soil and different aquatic habitats such as rivers, lakes, the open ocean, sewage and wastewater treatment plants (WWTPs) (Oyedara et al., 2016, Paix et al., 2019).

Predatory bacteria can be found within a broad taxonomy, including facultative and obligatory predators, defined by their feeding behaviours. While obligate predators survive by consuming prey cells, facultative predators readily switch to a saprophytic lifestyle, consuming a wide array of substrates in the absence of appropriate prey (Jurkevitch, 2007, Korp et al., 2016). To date, an obligatory predatory lifestyle is limited to α-proteobacteria (genus *Micavibrio*) and δ-proteobacteria (Families: *Bdellovibrionaceae, Bacteriovoracea, Peredibacteraceae*, *Halobacteriovoraceae* and *Pseudobacteriovoraceae*), all classified under the umbrella terminology *Bdellovibrio* and like organisms (BALOs) (Rotem et al., 2014, Paix et al., 2019).

Predatory bacteria are widely distributed, they are diverse, and they exhibit a variety of hunting strategies. Bacterial hunting strategies can be categorized into three general groups. In Wolf pack/ Group attack social bacterial predators, such as *Lysobacter spp*., and members of the Myxobacteria, like *Myxococcusxanthus*, tend to attack prey as groups, even if they are capable of doing so as isolated individuals. Such group predation can be accomplished remotely via the secretion of diffusible compounds that kill and decompose hapless neighboring prey (Velicer et al., 2009). The production of diffusible predatory compounds has profound social implications. The breakdown of prey cells by such secreted weapons creates a "public good" in the form of consumable nutrients from dead prey. Any nearby cell resistant to predatory lysis could potentially utilize this public good, even individuals that did not contribute to the kill (Mendes-Soares et al., 2013). In epibiotic predation, predators remain attached to the prey cell envelope while consuming the prey from the outside before dividing into two daughter cells (Jurkevitch, 2007, Pérez et al., 2016) They secrete enzymes directly into the interior of their victim and then assimilate hydrolyzed molecules from the interior of the prey cell. Examples include *Micavibrio, Bdellovibrioexovorus*, *Vampirovibrio* and *Vampirococcus* species which suck the cytoplasm out of bacteria. They can synthesize and secrete an unusual number of hydrolytic proteins, such as lipases, glycanases, peptidases, and proteases, which are probably involved in damaging and digesting prey cell structures. The third hunting strategy is endobiotic predation or direct invasion, in which an individual predatory cell secretes hydrolytic enzymes that perforate and modify the prey cell wall to penetrate either into the periplasmic space or into the cytoplasm (Rotem et al., 2014). Endobiotic predators can be distinguished with respect to whether they divide within the cytoplasm or periplasm of the prey (for example, *Daptobacte*r and *Bdellovibrio spp*., respectively). Among the obligate predators, *Bdellovibrio bacteriovorus* is by far the most studied BALOs.

6. BDELLOVIBRIO BACTERIOVORUS

B. bacteriovorus was accidentally identified in the 1960s and was described as a small parasite, and obligate predator of Gram-negative bacteria by Stolp and Starr (1963). It was further described as a highly motile, δ-proteobacterium that employs an endobiotic (periplasmic) hunting strategy which entails the invasion of, and proliferation within, the periplasm of Gram-negative bacteria. Bdellovibrios are ubiquitous in nature like other predatory bacteria and have also been recovered from the gills of blue crabs and oysters, and more recently from mammalian feces and the mammalian gastrointestinal tract (Rotem et al., 2014). All predators originally isolated were named *Bdellovibrio*, however, this taxonomy proved to be insufficient. Recent analyses of 16S rRNA genes revealed a wide genotypic diversity among these bacteria (Jurkevitch and Davidov, 2006).

6.1. Life cycle of B. Bacteriovorus

B. bacteriovorus consumes its Gram-negative bacteria prey in a process that typically lasts 3-4 h. Initially, *B. bacteriovorus* recognizes, attaches to, and enters the prey cell, reinforcing, traversing, and resealing its entry port. Although recognized as an obligate predatory bacterium, *B.*

bacteriovorus can switch to a host-independent lifestyle, demonstrating axenic growth on complete media. In the Attack Phase, collision with the Gram-negative prey cells occurs seemingly at random, and it has been suggested that the predatory cell remains reversibly attached for a brief "recognition" period before becoming irreversibly anchored (Lambert et al., 2016). It invades into a host by creating a pore in the outer membrane and crossing the peptidoglycan layer to establish itself within the prey periplasm finally. Successful recognition triggers the transition, as mentioned earlier, to an intermediate phase that facilitates invasion into the host cell and formation of an osmotically stable niche, protected from phage attacks and pollutants, called bdelloplast (Friedberg, 1977, Yair et al., 2009). It has been proposed that *B. bacteriovorus* uses its type IV pili to pass through the membrane, then sheds the flagellum and reseals the pore after entering the prey. Sensing of a second prey cue facilitates the transition to the Growth Phase and filamentous growth. Bdelloplast formation causes a distinct rounding up of the usually rod-shaped prey cells, resulting from peptidoglycan cell wall modifications. This modification has been shown to prevent self-competition between individual predators for the same prey and promote a 1:1 predator-to-prey ratio. When prey is exhausted, the predator is divided by septation into several flagellated progeny cells, followed by host cell lysis and progeny release, after which the cycle begins new (Lambert et al., 2015, Avidan et al., 2017).

7. POTENTIAL APPLICATIONS

Due to their unique lifestyle, BALOs have long been recognized as a potential therapeutic, water clean-up and biocontrol agent and source for discovering novel biotechnological tools for research (Yair et al., 2009, Perez et al., 2016). *Bdellovibrio bacteriovorus* is among the best-studied BALOs and serves as a model organism for bacterial predation. Its prey range includes several known human pathogens that either already has acquired or is at significant risk of acquiring resistance to antibiotics, such as enterohemorrhagic *Escherichia coli*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Pseudomonas*, and *Salmonella* (Dwidar et al., 2012a, Shatzkes et al., 2016). Predators such as *Bdellovibrio* and *Micavibrio* can kill human Gram-negative pathogens such as *Aeromonas*, *Bordetella*, *Burkholderia, Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Proteus*, *Salmonella*, *Serratia*, *Shigella*, *Vibrio*, *Yersinia*, *Helicobacter pylori* and *Legionella* that have acquired or are at risk of acquiring resistance to antibiotics. During the predatory lifecycle, the prey cell is killed in a short time (<30 min) (Rittenberg and Shilo, 1970), and therefore the prey would have to express means of defense quickly enough to resist predation, something not yet seen (Lambert et al., 2010).

Bdellovibrio is an aerobic predator, conditioning it to be an effective treatment in aerobic environments such as superficial burns or wounds, eyes, and lungs (Dwidar et al., 2012a, Shatzkes et al., 2016). Nevertheless, this predator can tolerate microaerophilic conditions, which widens its area of use to treat gastrointestinal and periodontal infections (Dashiff et al., 2011, Dwidar et al., 2012b).

7.1. BALOs proteases as novel antibody-modulating tools

The application of bacterial enzymes as biotechnological tools for antibody analysis has long been standard practice. *B. bacteriovorus* is now emerging as a new source for the identification of novel enzymes with biotechnological potential. This potential can be exemplified by identifying and characterizing BALOs Proteases (BspK and BspE) with described enzymatic activities on human antibodies (Bratanis et al., 2017, Bratanis and Lood, 2019). Currently approved therapeutic MAbs are mainly of subclass IgG1 (Irani et al., 2015) and are used primarily in the treatment of various forms of cancer (Scott et al., 2012) and autoimmune diseases (Hansel et al., 2010). However, product approvals of biological agents, such as antibodies, that are naturally heterogeneous and undergo a wide range of posttranslational modifications (PTMs) require rigorous controls to guarantee their quality and safety prior to any clinical application. PTMs may generate unwanted product variants. The current methodology used for such quality controls generally requires cleavage of the antibodies into smaller components to facilitate the analysis. BspK specifically hydrolyses IgG_1 (most common therapeutic antibody) on the hinge, enabling middle-down MS analysis of the biological therapeutics (Bratanis et al., 2017). Similar enzymes (e.g., Ides, SpeB) are currently being used within the biopharma industry for such purposes. BspE specifically hydrolyses the Fc-tail from IgA, with its glycan attached (Bratanis and Lood, 2019). While IgA is not commonly used to develop therapeutic antibodies, BspE is still a valuable tool for the basic research of IgA, Fc-interactions, and complement activation – findings that may eventually be translated into products. The rapid development and increasing number of approved mAb on the market creates a need and incentive to identify and characterize novel antibody degrading or modifying proteins.

7.2. Predatory bacteria as biocontrol agent

Biological control means the use of any organism to target an undesirable population of another, is a technique being increasingly recognized for its low cost and limited adverse effects on the environment, wildlife, and public health (Kergunteuil et al., 2016). In both natural and artificial habitats, contamination with microorganisms can

sometimes have detrimental outcomes. Where conventional methods for removing contaminating and/or pathogenic microorganisms fail, biocontrol agents might constitute a viable alternative. The idea of using predatory bacteria as a biocontrol agent is also gaining momentum. For example, poultry farming strategies to prevent salmonellosis include good agricultural practices combined with additional prevention measures to which predatory bacteria could potentially be added. It has been shown that orally administered *B. bacteriovorus* can effectively manage *Salmonella* infections in young chicks without adverse effects on the chick's health and well-being (Atterbury et al., 2011). It has also been proposed that *B. bacteriovorus* might be useful in the freshwater farming industry as a biological control agent of the shrimp pathogens *V. cholerae* (Cao et al., 2015) and *V. parahaemolyticus* (Kongrueng et al., 2017). BALOs also have several future applications in agriculture. Introduced or naturally occurring strains, cultured at a large scale, could be used as a wide-spectrum biocontrol agent combating phytopathogens that would otherwise damage the crops (Scherff, 1973, Jurkevitch et al., 2000, McNeely et al., 2017, Youdkes et al., 2020). The ensuing process of food spoilage might also be mitigated through predation. Saxon et al. (2014) showed that *B. bacteriovorus* could eliminate *Pseudomonas tolaasii*, a problematic pathogen of cultured mushrooms. Administration of *B. bacteriovorus* on the surface of post-harvest mushrooms resulted in reducing brown-blotch lesions, which could help extend the product's shelf-life. Saxon et al. (2014) and Ottaviani et al. (2019) demonstrated that *B. bacteriovorus* could control *E. coli* and other spoilage bacteria in meat products. This preliminary study showed that predatory bacteria could complement current methods of food spoilage prevention, as well as be a natural alternative to preservatives and antioxidants. It has also been suggested that predatory species can be used later on in the food manufacturing process to remove bacteria from processing equipment (Fratamico and Cooke, 1996). In light of the current energy crisis, research centered on micro algae-derived biofuel is picking up and, by many, considered a promising alternative. However, the growth of microalgae in open ponds is often affected by bacterial contamination. Li et al. (2018) demonstrated that a *Bdellovibrio* sp. limited the number of contaminating bacteria, thereby promoting microalgae growth and the production of green biofuel. Another pressing environmental and economic concern is the amount of "waste activated sludge" generated by Waste water treatment plants. Waste activated sludge is the excess microorganisms that need to be removed to maintain balance within the biological system. Several studies have indicated that bacterial predation, in combination with environmental factors such as regulation of dissolved oxygen concentrations, is crucial in limiting waste-activated sludge production. It has been demonstrated that the treatment of activated sludge with *B. bacteriovorus* effectively improved it's de-water ability in a dose-dependent manner (Yu et al., 2017).

7.3. Biofilm formation and degradation by BALOs

Biofilms form when bacteria adhere to surfaces and excrete a glue-like extracellular polymeric substance that protects and anchors them to materials and tissue. One of the significant difficulties in controlling surface-attached bacteria is their enhanced resistance to antimicrobial agents – biofilms can be up to 1000 times more resistant to antimicrobial agents than their planktonic counterparts. Though being an exclusive predator of Gram-negative bacteria, even Gram-positive biofilms are prone to degradation by BALOs. Through a plethora of secreted enzymes, particularly its proteases and nucleases, *B. bacteriovorus* can both inhibit the formation of and reduce preformed biofilms of Gram-positive bacteria (Monnappa et al., 2014). *B. bacteriovorus* can attack and reduce existing *E. coli* and *P. fluorescens* biofilms (Kadouri et al., 2005). *M. aeruginosavorus* can attack lab strains and numerous clinical isolates of *P. aeruginosa* biofilms (Kadouri et al., 2007). *M. aeruginosavorus* also can attack the biofilms of both *K. pneumoniae* and *Burkholderia cepacia* in liquid culture.

Of particular interest is the ability of BALOs to disrupt biofilms of medically relevant pathogens (Sun et al., 2017), as well as the possibility to use them synergistically with certain antibiotics like ciprofloxacin (Chanyi et al., 2016).

7.4. Predatory bacteria as a strategy to combat horizontal gene transfer (hgt)

The concept of elimination of recombinant DNA from the environment by using predatory bacteria was studied earlier by Monnappa et al. (2013). They demonstrated that *B. bacteriovorus* HD100 could effectively remove recombinant bacterial strains in aqueous and soil slurry environments. This, in turn, led to a reduction of the prey-associated recombinant plasmid, limiting the chances for HGT. Predatory bacteria thrive in environments with high prey density and naturally occur in WWTPs (Feng et al., 2016, Yu et al., 2017). Not only do they kill the prey, but they also wholly degrade its DNA (Matin et al., 1972, Rosson et al., 1979), consequently reducing the pool of ARGs in the environment. The repertoire of enzymes secreted by this bacterium may further contribute to the reduction of the HGT. Extracellular proteolytic and nuclease activities have been demonstrated in *B. bacteriovorus* HD100 cultures (Bratanis et al., 2017, Bratanis et al., 2019). Thus, hypothetically, nucleases released into the environment may contribute to eliminate the "cell-free ARGs," while extracellular proteases may act on phage particles, leading to their inactivation.

8. CONCERNS

While biotechnological application of purified enzymes from predatory bacteria seems realistic, the application of the whole cells is likely to be more challenging. Several environmental factors such as optimal growth conditions, pollutants, or microbial interactions must be considered before natural enemies can be used as a biocontrol agent in complex systems. Bdellovibrios are very sensitive to various environmental pollutants, affecting their predatory activity (Wehr et al., 1971, Varon et al., 1981, Markelova, 2002). Despite preying on a wide range of host bacteria, BALOs might behave differently in mixed microbial communities. The agricultural application of BALOs can be affected by herbicides that are widely used for weed control. Wehr et al., 1971 evaluated the effect of 17 different herbicides for activity against *B. bacteriovorus*. The plaque formation was inhibited, to various degrees, by 11 herbicides included in the analysis.

Finally, the possible emergence of HI mutants of *B. bacteriovorus* might be problematic for biotechnological applications. They not only exhibit reduced predation ability but may also contribute to the formation of undesired biofilm. The potential use of live bacteria as therapeutics raises concerns regarding the safety and efficacy of BALOs administration. This aspect has been, and continues to be, thoroughly investigated using both human cells and numerous animal models such as zebra-fish, mice, rats, rabbits, guinea pigs, and chicks. The results demonstrate an inability of *B. bacteriovorus* and *M. aeruginosavorus* to invade mammalian cells and no apparent pathological effects or signs of cytotoxicity or reduction in cell viability, supporting the proposition that these two BALOs are inherently non-pathogenic to mammals (Gupta et al., 2016, Willis et al., 2016, Shatzkes et al., 2017a). Another concern regarding the applicability of *Bdellovibrio* as therapeutics is the development of prey resistance and incomplete eradication of prey. Nevertheless, it has been argued that despite the display of some prey resistance, the number of resistant preys is considerably low because these predators are not antibiotic producers, and their killing mechanisms do not target specific receptor proteins that can evolve resistance (Sockett et al., 2004, Jurkevitch, 2007). Topical application, ingestion, injection, or intranasal inoculation of whole cells have no apparent cytotoxicity, neither in terms of pathological effects, nor in terms of diminution in cell viability in *in vitro* cell culture models (Shanks et al., 2013, Gupta et al., 2016, Monnappa et al., 2016) or *in vivo* animal models (Shatzkes et al., 2015, Romanowski et al., 2016, Shatzkes et al., 2017b, Shatzkes et al., 2017c). Also, Predatory bacteria do not incite a systemic or sustained immune response, most likely due to the special structure of their lipopolysaccharide, which lacks the typically negatively charged phosphate groups, resulting in only low binding affinity to the lipopolysaccharide receptors in human immune cell pathogens (Willis et al., 2016).They are passively engulfed by macrophages and can persist inside these cells over 24–48 h as non-replicative forms, although they retain their predatory competence. Although they do not affect host cell viability, they stimulate moderate cytokine responses (Shanks et al., 2013, Shatzkes et al., 2015, Gupta et al., 2016, Monnappa et al., 2016). Predator persistence inside macrophages for sufficient time to prey on pathogens opens the way to using predatory bacteria to eliminate intracellular pathogens, such as *Salmonella, Klebsiella*, or *Francisella* species.

Moreover, the relatively benign occupancy of macrophages by *Bdellovibrio* could prevent other intracellular pathogens from entering. However, this passive uptake suggests that macrophages might present predatory bacteria to antibody-forming cells, and consequently, humans could develop immune reactions against them after repeated exposures (Madhusoodanan, 2019), suggesting that the predatory treatment could be used only once. The natural resistance of Bdellovibrio to βlactam antibiotics also opens up the possibility for treatments using these bacteria in conjunction with penicillin (Sockett et al., 2004, Dwidar et al., 2012b). One of the main advantages of using these predators as therapeutic agents is the failure of prey bacteria to develop induced resistance against predation. The main reason for this is that these predators are not antibiotic producers, and their killing mechanisms do not target specific receptor proteins that can evolve resistance.

Using live bacteria as antibacterial therapy poses manufacturing and regulatory challenges. New standards will have to be created for predatory bacteria-based remedies because they that do not fit the guidelines for chemical drugs.

9. CONCLUSION

The rampant rise of antibiotic resistance has led us to explore and further study unconventional therapies as an effective alternative to antibiotics. Research on the use of predatory bacteria as living antibiotics yields promising results, and although more research is required to demonstrate their efficacy *in vivo*, they represent a serious alternative to be considered. Further work is needed to evaluate the dissemination of predatory bacteria from the administration site and determine any long-term effects of exposure on the host or their resident microbiota.

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