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Predatory prokaryotes wage war against eye infections

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Predatory bacteria

Predatory bacteria, bacteria that prey upon other bacteria, are gaining interest as a potential therapeutic tool to combat infections. Recent reviews cover what is known about the biology and potential application of these organisms [1–3]. The focus of this article is to discuss the potential use of predatory bacteria to control ocular infections. The most studied predatory bacterium to date is *Bdellovibrio bacteriovorus*, a small, highly-motile, Gram-negative bacterium that preys on other Gram-negative bacteria. This bacterium has an extraordinary life cycle in which it collides with its prey, burrows through the bacterial membrane, divides into several daughter cells, and lyses the host bacteria to start a new round of predation [3]. Other predatory bacteria, such as *Micavibrio aeruginosavorus* may also have medical potential [4–6]; however, *B. bacteriovorus* will be discussed here as it is the most thoroughly studied.

Efficacy against major pathogens

Recent studies have shown that *B. bacteriovorus* is capable of killing a broad range of human pathogens and opportunistic pathogens [4] including multidrug-resistant hospital isolates of *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [5]. Predatory bacteria are able to effectively prey upon bacteria in biofilms [7], which are notoriously difficult to treat due to antibiotic tolerance.

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Question of safety

It is easy to imagine using these organisms to treat unwanted pathogens in aquatic and industrial settings; however, the use of these predators to treat human and animal infections is another question. Are these organisms safe? The strongest evidence to support safety in humans are findings reported by Iebba *et al.*, in which *B. bacteriovorus* was found in the gut of healthy individuals [8]. Earlier work highlighted the fact that *Bdellovibrio* had a unique lipid A portion of its lipopolysaccharide structure that was much less immunogenic than typical lipopolysaccharide [9]. A recent review by the Mitchell group covers what is known regarding safety of these predatory bacteria [1], which demonstrated that multiple studies failed to detect deleterious effects following topical application, ingestion or injection of *B. bacteriovorus* into vertebrates. This includes a recent study conducted at the University of Nottingham (UK) that documented no negative health effects on chickens, which received oral treatments of *B. bacteriovorus* [10]. While these studies are limited, they do positively support the notion that predatory bacteria may be part of future therapeutic strategies.

Potential use on the ocular surface

It would seem that predatory bacteria would have its greatest success as an antimicrobial or adjuvant to be used alongside antibiotics for topical infections such as those of the skin or other mucosal surfaces. Ocular surface infections such as bacterial conjunctivitis and microbial keratitis have a large cost to society and are a major source of vision loss. These infections are often caused by Gram-negative bacteria such as *Haemophilus influenzae*, *P. aeruginosa*, and *Serratia marcescens*; the latter two have been verified as being susceptible to predation by *B. bacteriovorus* [6].

B. bacteriovorus has been reported to be resistant to β -lactam antibiotics, so it is conceivable that combination therapy using both predatory bacteria and antibiotics could be used [2]. In addition to antibiotics, the combination of using predatory bacteria with biofilm-degrading enzymes or phage was also brought forward [11, 12]. Unlike antibiotics or the use of bacteriophage to kill bacteria, no stable genetic resistance of host bacteria to *B. bacteriovorus* has been identified, despite attempts to isolate resistance [13]. Additional attempts to enrich for prey-resistant phenotypes by culture enrichment or mutagenesis also failed [Kadouri DE, [Pers.Comm.](#)]. An important theoretical advantage of predatory prokaryotes over antibiotics is that if host bacteria did evolve resistance, then there will be equivalent selective pressure for predatory bacteria to evolve mechanisms to overcome any resistance mechanisms acquired by its host bacteria.

Review of the literature

The original paper relating to the potential of *B. bacteriovorus* in treating eye infections dates from 1972 and remains the only paper to test *B. bacteriovorus* in an ocular model *in vivo* [14]. In this paper, Nakamura showed that *Shigella flexneri* eye infections could be prevented, by co-inoculation with *B. bacteriovorus* [14]. Instillation of *B. bacteriovorus* at 12, 48 and 72 h after *S. flexneri* inoculation was tested and time-dependent prevention of keratoconjunctivitis was observed, with apparent full protection at 12 h and little protection by 72 h. At first glance, this suggests that the predatory bacteria efficiently killed the *S.*

flexneri bacteria; however, in the same study, the co-inoculation of nonpredatory bacteria *Escherichia coli* was equally as effective in preventing *S. flexneri* infections. Therefore, it is not clear whether predation had any impact on the reduced virulence of *S. flexneri* in the study. An important outcome of this study was that no adverse affect was described when 10^9 *B. bacteriovorus* were applied topically to rabbit eyes, supporting that they do not induce a strong inflammatory response.

The second paper aligning predatory bacteria and ocular infections, used an “*in vitro* model of infectious keratoconjunctivitis to assess *Moraxella bovis* pathogenesis” [15]. The use of ‘keratoconjunctivitis’ could be considered to be an overstatement as the mammalian cells used in the model were bovine kidney cells and the study was performed *in vitro*. Nevertheless, *B. bacteriovorus* provided some protection to the mammalian cells against *Mycobacterium bovis* under the conditions used in this study.

A third study used two strains of *B. bacteriovorus* and one *Micavibrio* strain to test whether they could prey upon common ocular bacterial pathogens and to determine whether predatory bacteria have deleterious effects to a human corneal cell line. This *in vitro* study demonstrated that predatory bacteria can kill human ocular pathogens *in vitro*, are not cytotoxic to human corneal limbal epithelial cells, and do not induce proinflammatory markers (IL-8 and TNF- α) from human corneal limbal epithelial cells [5].

Challenges

Potential limitations to the use of predatory bacteria for ocular infections include: limited host range; susceptibility to host defense and antibiotics; and potential allergic reactions. Reported predatory bacteria cannot prey upon Gram-positive bacteria, which is a major problem as Gram-positive bacteria are a major cause of ocular infections [16]. In one recent publication, Monnappa *et al.* showed that *B. bacteriovorus* proteolytic enzymes can prevent biofilm formation by *Staphylococcus aureus* [12]. While it is unlikely feasible to treat infections with strains that over-express proteases as it might have a negative effect on the surrounding tissue, this study serves as a proof of principle that the native enzymes produced by *B. bacteriovorus* could be manipulated for biocontrol of Gram-positive bacteria. In fact, the genomes of predatory bacteria are replete with genes predicted to code for hydrolytic enzymes evolved to digest its bacterial host [17, 18]. Another drawback of using predatory bacteria to control infection is, like other predators in nature, the predator never eradicates all of its prey from its environment. Although this might be seen as a major obstacle, the predator might still be able to clear the bulk of the infection allowing the immune system to deal with the residual pathogens. Predatory bacteria could also be used in sync with other antimicrobial therapies such as phage or antibiotics, rendering them more efficient and allowing total removal of the infection. An additional obstacle is that the human ocular surface is an inhospitable place for microbes due to innate immune defenses [19]; therefore, the use of predatory bacteria as a probiotic might not be feasible. This is because the predatory bacteria may be killed by the immune system and because there is a limited food supply for predatory bacteria on the ocular surface [20]. However, as the relationship between predatory bacteria and the immune system was not investigated, one could argue that a poorly inflammatory *Bdellovibrio* would not provoke an immune response and will

not be rapidly cleared from the site. Lastly, it is conceivable that certain patients would develop allergies to predatory bacteria that could impact their widespread use as therapeutics.

Conclusion

The existing literature on the use of predatory bacteria to treat ocular infections, or indeed any infections, is lacking *in vivo* studies specifically designed to evaluate the ability of predatory bacteria to treat ocular infections without toxicity and excessive inflammation. Despite the weaknesses of predatory bacteria, the lack of stable resistance by opportunistic pathogens to *B. bacteriovorus* makes them an attractive potential therapeutic.

Future perspective

As the medical community is facing a new area of drug-resistant pathogens, the need for new therapeutics and approaches to control infection is never more urgent. In the last few years, substantial progress in our understanding of the biology and genetics of predatory bacteria has been made. Additionally, our understanding of the ability of predatory bacteria to control human pathogens and biofilms *in vitro* has improved. We believe that in the next few years, work will be conducted with the aim of better understanding the mechanisms involved in predation and, most importantly, what governs prey specificity. *In vivo* studies will be conducted with the goal of assuring that predatory bacteria are harmless to the host and to test the efficacy of using predatory bacteria to control infection. Finally, we speculate that for predatory bacteria to be successfully used as live antibiotics, they will need to be coupled with other therapeutics to ensure that resistance does not develop and to enhance their effectiveness as suitable adjuvants for our limited arsenal of antibiotics.

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