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Predatory bacteria: Living Antibiotics, Biocontrol Agents, or Probiotics?

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Abstract

Predatory bacteria seek out and kill other bacteria for food. These predators have been hypothesized to be useful "living antibiotics." Here, we discuss the applications for which these bacteria have been used. Recent data suggested predators are prevalent in the environment and can even be isolated from the human GI tract. These studies have prompted us to discuss the logical applications as well as the terminology associated with the use of these remarkable organisms. It has been hypothesized many times that these bacteria could be the key to developing novel treatments and now more than ever we need to investigate these applications in light of the rise in multi-drug resistance among medically important bacteria.

For seven decades humankind has benefited from the availability of antibiotics to treat bacterial infections. Antibiotics are critical because vaccines are not available for prevention of all infectious diseases. In September 2013, the Centers of Disease Control (CDC) issued a report titled "Antibiotic Resistance Threats in the United States, 2013" which is the first extensive report to prioritize the threat levels of antibiotic resistant bacteria. It is estimated by the CDC report that in the US, two million people a year experience illnesses caused by antibiotic-resistant bacteria and that these infections result in 23,000 deaths. Two other unfortunate facts make this report even more daunting: 1) there are very few prospective new antibiotics in development and 2) pharmaceutical companies are greatly reducing research and development of new antibiotics. Collectively, these scary realities mean that if new solutions are not identified in the near future, infectious diseases could once again return to prevalence levels that existed before the antibiotic era.

What are the potential solutions to the antibiotic resistance problem? A classic proverb says, "The enemy of my enemy is my friend." Before penicillin, the initial efforts to treat diseases focused on these enemies. At the Pasteur Institute, bacteriophages were discovered in 1917 and these bacteria-infecting viruses were used to treat an array of infections with considerable success (1). After the dawn of the antibiotic era, a new class of "enemies" of bacterial pathogens was discovered in the 1960s: predatory bacteria. *Bdellovibrio bacteriovorus* is a predatory bacterium that invades the periplasm of Gram-negative bacteria, replicates, and finally lyses the host cell (2). *Bdellovibrio*-and-like-organisms (BALOs) have been shown to prey upon and kill a broad spectrum of Gram-negative bacteria. Since their discovery, predatory bacteria have been hypothesized in many publications to be useful as "living antibiotics" (2, 3). However, relatively few real world applications have been described. The forefathers of the predatory bacteria field experienced difficulties in funding their research and their efforts were hampered by the emergence of molecular biology (4). However, recent publications are re-vitalizing the field. In this review, we will provide illustrative studies that suggest the key to developing applications is to identify permissive environments for predation and in the future take advantage of genetic tools to engineer predators to not only be enemies of our enemies, but to be precise, ruthless killers of bacterial pathogens.

Origin of bacterial predators: discovery and early studies

The original discovery of *Bdellovibrio* occurred fortuitously while looking for phage in soil samples (5). Early studies from 1963 to 1973 focused on the mechanism of predation *in vitro* and the basic characterizations of *Bdellovibrio*. The first biocontrol application was the use of *B. bacteriovorus* strain, Bd-17 to control soybean blight caused by *Pseudomonas glycinea*, now known as *Pseudomonas syringae* pathovar *glycinea* (6). The predator could prey upon the soybean pathogen at a high rate to block systemic infection of the plant. Since this initial study was carried out to test the use of these types predators as biocontrol agents in plants, no studies have revisited these findings.

Bdellovibrio have been isolated from other reservoirs, including river and sewage water. *Bdellovibrio* was identified in high concentrations in water contaminated by Gram-negative pathogens and correlated with decreased bacterial loads in these samples (7, 8). In both studies it was proposed that *Bdellovibrio* participated in sewage purification and could be used to increase sewage degradation. However, it is not clear if this application was ever implemented.

Identification of *Bdellovibrio* in sewage has caused some confusion about the original identification of another predator, *Micavibrio aeruginosavorus*. *M. aeruginosavorus* is a Gram-negative obligate predatory bacterium that does not invade its prey like *Bdellovibrio* but rather feasts upon it by attaching to the outer-membrane (9). The original manuscripts that identified *Micavibrio* are in Russian. English translations improperly stated that *Micavibrio* was also isolated from sewage. Instead *Micavibrio* was actually isolated from storm drain water in Pushchino, Russia (10). In addition to the misinterpretation about sewage, the title of the publication has also been mistranslated. The English translation title on PubMed, indicates that *Micavibrio* preys upon Gram-positive organisms (10). Reading of the manuscript in Russian confirms that this has been mistranslated and no such data about *Micavibrio* predation of Gram-positive organisms was presented in the publication (10). *Micavibrio* has only been shown to prey on Gram-negative pathogens including *Pseudomonas aeruginosa* and *Escherichia coli* (11). The genome of *Micavibrio* was recently sequenced and analyzed (9). *Micavibrio* is particularly interesting because unlike *Bdellovibrio*, it cannot be cultured without prey and *Micavibrio* is an exceptional predator of *P. aeruginosa* (11-13) (Figure 1). *Micavibrio*'s lifestyle as obligate predator greatly hinders the study of this microorganism and future studies will have to determine better culture conditions and how to genetically engineer this bacterium.

A recent study looking at *Nitrospira* species has identified *Micavibrio*-like organisms in activated sludge from a wastewater treatment

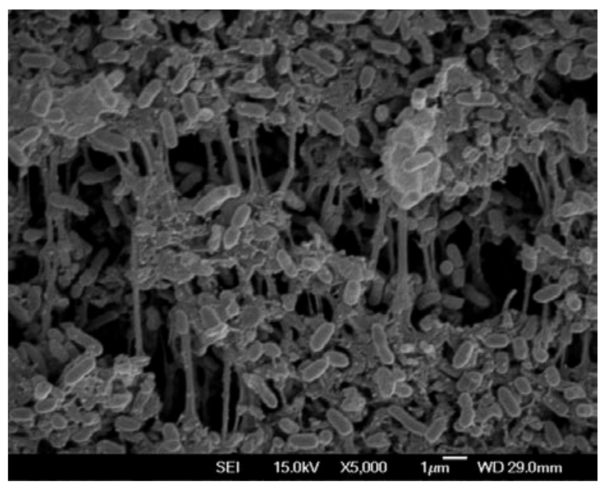

Figure 1. *M. aeruginosavorus* predation on *P. aeruginosa*. Scanning electron microscope image showing exopolysaccharide produced by *P. aeruginosa* in co-culture with *M. aeruginosavorus*. Exopolysaccharide matrix, microvesicles, and small *M.aeruginosavorus* cells were observed. The two bacteria were cultured at 25°C shaking at 180 rpm in ¼ strength Lysogeny broth (LB) for 48 hrs.

plant (14, 15). In light of all the metagenomics projects that are currently in progress, we suspect that more DNA sequences of predatory bacteria will be detected in the near future. These types of studies will help identify new ecological niches for predatory bacteria and answer the questions: “Where do the predators come from? Are the predators from the environment? Or do they reside in the GI tract and as a result are isolated from wastewater?” In another recent publication, *Micavibrio* species were found to prey upon *Vibrio parahaemolyticus* and *Vibrio vulnificus* in seawater and in oysters (16). *Micavibrio* has a role in the environment but can it survive in a human or be used as a living antibiotic as it has been proposed?

From the environment to the host

Aeromonas hydraphila is a common fish pathogen and can cause disease in humans as well. Two studies have indicated that *Bdellovibrio* can be used to treat *A. hydraphila* infection in fish (17, 18). *Bdellovibrio* strain BbC-1 was capable of preying upon 20 different isolates of *A. hydraphila* that infect fish, eels, crabs, mussels, and turtles (17). Furthermore, strain BbC-1 also preyed upon other Gram-negative fish pathogens (17). Aqua farming is a growing industry due to overfishing of the sea and freshwater bodies, and the use of *Bdellovibrio* as a prophylactic or treatment of *A. hydraphila* contamination could have major positive impacts on this industry.

In a groundbreaking study, the *B. bacteriovorus* type strain HD100 was shown to be a useful biocontrol agent against *Salmonella enterica* in young chickens (19). In this study, HD100 was administered to chicks with antacids to improve *Bdellovivrio* survival in the stomach and facilitate gut colonization. When this was performed on uninfected chicks, the overall diversity of the microbiota of the chicks gut was analyzed. Administration of *Bdellovibrio* decreased the diversity of cultivable microbiota of the gut but no adverse effects on the well-being of birds were observed (19). Next, HD100 was used to treat *Salmonella* infected chicks. As a result of predator treatment, reduced *Salmonella* numbers, as well as reduced abnormalities and inflammation in cecal morphology, were observed indicating *Bdellovibrio* treatment was beneficial for the chickens (19).

The study of *Bdellovibrio* treatment of *Salmonella* infections in chickens (19) has paved the way for therapeutic uses of predatory bacteria. However, it also brings up the question, should we call them living antibiotics or biocontrol agents? The original definition of antibiotics was used to define compounds secreted from living organisms. This differentiated these compounds from the chemicals or chemotherapies that were used at the time. Since predatory bacteria themselves are living and they are the agent that is responsible for killing the target or pathogenic bacteria, it is our opinion that the therapeutic uses of predatory bacteria to kill target pathogens should classify them as biocontrol agents.

Bacterial predators in humans: a new battlefield?

The effect upon the diversity of the gut of chicks builds a hypothesis that the GI tract may be a permissive location for the application of predatory bacteria. Furthermore, we now appreciate that human health is greatly affected by our gut microflora. One study identified the presence of *Bdellovibrio* in a single human fecal sample (20) which was an interesting finding in relation to the presence of predators in sewage as discussed above. In an extensive study, *Bdellovibrio* has been shown to be in higher prevalence and abundance in the GI tract of healthy humans (21). Samples from patient groups with inflammatory bowel, celiac, and cystic fibrosis diseases, were analyzed and compared to healthy individuals and, remarkably, healthy individuals harbored more *Bdellovibrio* predators. The study went on to look at the localization of *Bdellovibrio* in the small intestines and found that it was in higher abundance in the duodenum (21). These data correlated with observations showing that predation is optimal and preferential in aerobic conditions (7, 22). Various authors have hypothesized that *Bdellovibrio* could be considered as a probiotic (3, 21, 23). It will be interesting to see if predatory bacteria can be used to improve human health through the gut microbiota. Currently, the Human Microbiome Project is continuing to determine the composition of the human body by metagenomics (24) and it will be interesting to see if predatory bacteria such as *Micavibrio* or *Bdellovibrio* are found in the Project.

While predatory bacteria have been proposed to be useful therapeutics, very little work has addressed the potential caveats. *Bdellovibrio* has been found in the GI tract (21); however, we wonder if predatory bacteria could survive the various responses of the human immune system that are activated during a bacterial infection. *Bdellovibrio* strains seem to be very sensitive to antibiotics (25), likely because they do not come in contact with them in their natural habitat. In addition to these concerns, there will always be a societal issue to using bacteria to treat infections, especially in today's world where all bacteria are thought of as "dangerous". Much work is needed to demonstrate if predators can be used safely.

In this article, we have briefly described the uses of predatory bacteria as living antibiotics, biocontrol agents, and probiotics. However, there is skepticism as to the value of these claims and unfortunately there have been too few studies to validate that argument either way. In a recent study, *Escherichia coli* was engineered to seek out *P. aeruginosa* and deliver an antibacterial, thus killing the pathogen (26). While studies have clearly shown that predatory bacteria seek out prey and can kill them, the next phase of research for this field should be to improve the inherent predatory nature of these organisms. The genomes of *Micavibrio* and *Bdellovibrio* have been sequenced and analyzed (9, 14, 27) and genetic manipulation of *Bdellovibrio* has been established (28) which has resulted in a recent increase in publications. We proposed that it is time for researchers to revisit the groundwork that was laid over the past half century and devise clever applications to utilize predatory bacteria to address the emerging problems with multi-drug resistant bacteria. At the same time, it is important to return to the field and isolate new predators using our current problematic bacteria as bait. These exciting little predators have much to teach us and it is time for us to put our enemies' enemies to work solving our multi-drug resistant problems.

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