

Periodontal Predators Preying The Pathogens

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ABSTRACT:

Bdellovibrio are naturally occurring small, motile, predatory Gram negative bacteria that attacks and consumes other pathologic gram negative bacteria including periodontal pathogens like *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. They could remove biofilms and also have the ability to attack periodontal pathogens even in the presence of saliva. This article focuses on the life cycle of *Bdellovibrio* and the proven preying capacity of these bacteria in invitro studies showing a promising alternative modality for using them as a living antibiotic for the prevention and treatment of periodontitis.

Key words: Bdellovibrio, predatory bacteria, periodontitis.

INTRODUCTION

Periodontal diseases are multifactorial infections elicited by a complex of primarily gram negative bacteria that interact with host tissues and lead to the destruction of periodontal structures.¹ These bacteria persist in complex microbial communities attached to surfaces or associated with interfaces known as biofilms. These biofilms can be 1000-1500 times more resistant to antimicrobials than their planktonic counterparts.² Tissue invasive periodontal pathogens like *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Tannerella forsythia* are often difficult to eliminate with different antibiotic regimens showing limited success.

Predation is an interaction between predator and prey where a predator (an organism that is hunting) feeds on its prey (an organism that is attacked). *Bdellovibrio Bacteriovorus* is a gram negative, obligate aerobic bacteria which preys on pathologic gram negative bacteria, thus acting as a predator in preying the periodontal pathogens like *Aggregatibacter actinomycetemcomitans*. *Bdellovibrio* and like organisms called as BALO are obligatory predators that have the capability to access extremely thick biofilms. They could remove biofilms and also have the ability to attack periodontal pathogens even in the presence of saliva.

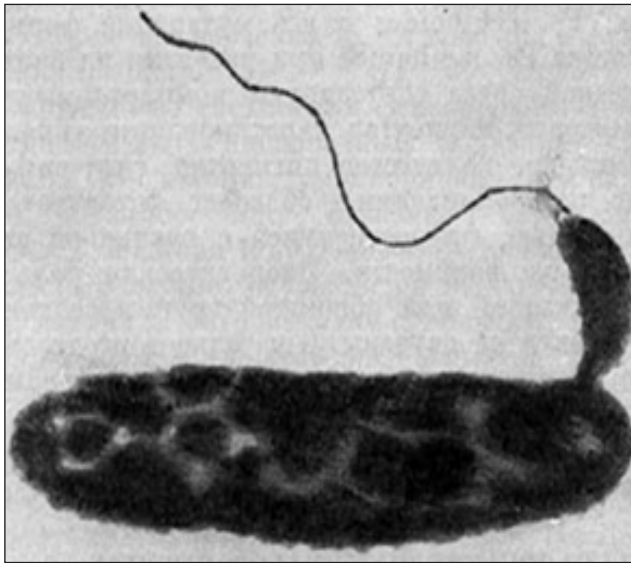
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It is motile by means of single, polar, sheathed flagellum. The size of *Bdellovibrio* varies from 0.2-0.5µm in diameter and 0.5-1.4 µm in length.³ It feeds only on range of pathogenic gram negative bacteria, and not on mammalian and human cells.⁴ The basis for host specificity is unknown, a particular *bdellovibrio* strain may parasitize a broad or narrow range of gram- negative species.

Bdellovibrio

Scientific Classification⁵

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Deltaproteobacteria
Order:	Bdellovibrionales
Family:	Bdellovibrionaceae
Genus:	<i>Bdellovibrio</i>
Species:	<i>bacteriovorus</i>



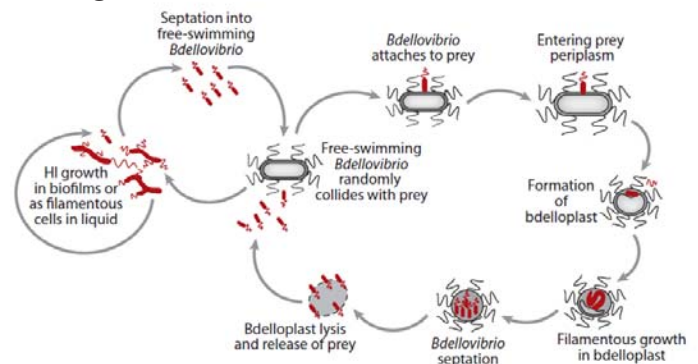
HISTORICAL PERSPECTIVE

Bdellovibrio bacteriovorus is a small, curved Gram negative predatory bacteria discovered by chance in 1962 by Stolp and Petzold while they were trying to isolate bacteriophages for plant pathogenic bacteria from soil.⁶ Since then they are found to be widely distributed in aquatic and terrestrial environments. The term *Bdellovibrio* was coined by Robert E. Buchanam, a noted taxonomist and

Professor as it describes the morphology and way of living of this bacteria i.e. the curved appearance of this bacteria and the way they stick to their prey to absorb the prey cell content like a leech (*Bdella* in Greek).

Life Cycle Of Bdellovibrio Bacteriovorus

The predatory life cycle of *Bdellovibrio* lasts around 34 hours and has several stages which are microscopically distinct. It exhibits a biphasic life cycle alternating between a free living, motile, predatory or hunt phase and an intraperiplasmic growth phase. In hunt phase, *Bdellovibrio* are free living and they search for a suitable host, but do not multiply. It randomly collides with and attaches to the host outer surface. It penetrates the host periplasm, multiply in their cytoplasm and finally burst their cell envelopes to start once again. The life cycle of *Bdellovibrio* can be divided into various stages-



Life Cycle of *Bdellovibrio bacteriovorus*
(Renee 2009)⁷

1. Prey Location

The mechanism by which the *Bdellovibrio* differentiates between a gram negative, gram positive bacteria or inanimate particle is unknown. It attaches to both gram positive and negative bacteria, but somehow detects in a few minutes, whether it is attached to a living gram negative cell, and if so invades or, if not, leaves.⁸ Chemotactic attraction to compounds produced by prey cells might be important in prey location and flagellar motility may be involved in prey penetration. They move with high velocity of about 160µm/sec or over 100 times their length per sec and collide with their prey. To retain

this high metabolic activity and to fulfill its requirement for organic substrates for growth, this *Bdellovibrio* have to quickly find its prey to avoid starvation and death.⁹

*Lambert et al*⁷ showed that *B. bacteriovorus* 109J with a mutation in one of over 20 predicted methyl- accepting chemotaxis protein genes of *Bdellovibrio* significantly reduced, but did not abolish, predatory efficiency in liquid cultures.

2. Attachment

A host cell encounter is an apparently random event, where rapidly swimming parasite collides with and attaches to the host outer surface. After *Bdellovibrio* collision with its prey it gets attaches to its prey reversibly for short duration and then gets anchored to its prey irreversibly using appendages located at the non flagellated pole. This takes place within minutes.

3. Penetration

The mechanism for penetrating the host outer membrane is unknown. For penetration the prey cytoplasmic membrane should come in contact with the invading pathogen. After *Bdellovibrio* irreversibly attaches to the prey, it creates a pore in the host envelope for its entry in about 10 minutes.¹⁰ Penetration of the host peptidoglycan is thought to be caused by *Bdellovibrio* directed enzymatic activities.¹¹ It progress from free living attack phase to the intracellular growth phase. As *bdellovibrio* enters the prey periplasm, the flagellar motility stops and then it sheds its flagellum.

The *Bdellovibrio* causes the cessation of metabolic activity within the host cell. The host cell loses the ability to synthesize protein, RNA and DNA and can no longer respire exogenous substrates and its cytoplasmic membrane becomes permeable to small molecules.

4. Establishment

Once *Bdellovibrio* is inside the periplasm, it seals the membrane hole through which it gains its entry and the host cell is now called a bdelloplast. Within bdelloplast the invader possess a protected niche for growth and reproduction.

5. Elongation

Represents the actual growth phase inside the bdelloplast. The predator reutilizes the existing prey channel proteins to achieve access.

6. Septation And Development

The *Bdellovibrio* detect that the prey cytoplasm is exhausted, and several uniformly shaped mononucleate daughter cells of normal size are formed. Production of lytic enzymes are initiated and daughter cells equipped with a flagellum are produced. The growth of *Bdellovibrio* is detectable through an increase in the respiration rate and the incorporation of host derived nucleotides into *Bdellovibrio* DNA and RNA.¹²

7. Release

The predator produce hydrolytic enzymes that degrade the modified peptidoglycan layer from inside and multiple *Bdellovibrio* are released from the bdelloplast. This marks the transition from the intracellular growth phase to the free living attack phase.

ADVANTAGES

1. *Bdellovibrio bacteriovorus* are unable to infect mammalian cells (*Simpson, 1972*).¹³ They are regarded as safe since no infectious diseases or pathogenic effects have been associated with them.
2. They have the ability to invade and penetrate biofilms and destroy them and also have the ability to attack periodontal pathogens even in the presence of saliva (*Dashiff et al, 2011*).¹
3. *Bdellovibrio* lipopolysaccharide does not induce a strong immunological response (*Schwudke et al, 2003*).¹⁴
4. The initial dose of predator can be low since this organism multiplies rapidly as it feeds.

LIMITATIONS

These bacteria are strict aerobes, so their use may be limited in anaerobic environments like deep periodontal pockets. But according to the study done by *Schoffield et al (1996)*,¹⁵ BALO strains could survive for up to nine days under anoxic environments and some BALO strains could grow and attack under microaerobic conditions.

DISCUSSION

Several studies have been published describing the possible use of BALOs as a predator for oral pathogens. Most of the pathogenic bacteria in periodontitis are gram negative and so susceptible to BALO, in contrast to the protective gram positive bacteria, which are resistant to BALO predation. Various studies have been conducted on the susceptibility of *Aggregatibacter actinomycetemcomitans* by *Bdellovibrio*. **Van Essche et al (2009)¹⁶** conducted an invitro study to find whether *Bdellovibrio bacteriovorus* can attack and kill the periodontal pathogen *Aggregatibacter actinomycetemcomitans* and concluded that *A.actinomycetemcomitans* is a prey for *Bdellovibrio bacteriovorus* HD100 both in planktonic and in a biofilm state. **A.Dashiff and D.E.Kadouri (2011)¹** conducted a study to examine the ability of predatory bacterium *Bdellovibrio bacteriovorus* 109J to prey on bacteria associated with periodontal diseases and concluded that *Bdellovibrio bacteriovorus* 109J prey on *Aggregatibacter actinomycetemcomitans*. *Bdellovibrio* have the capability to access extremely thick biofilms. They could remove biofilms and also have the ability to attack periodontal pathogens even in the presence of saliva. **Nair Rohan et al (2011)⁴** in their invitro study concluded that *Bdellovibrio bacteriovorus* have the ability to reduce an existing biofilm in about 2 hrs only with maximum reduction after 6 hrs. **Daniel Kadouri and George A. (2005)¹⁷** in their study concluded that *B. bacteriovorus* have the capability to access biofilms as thick as 30 µm and is not restricted to the surface of the biofilm. Thus they can penetrate the biofilm and kill the pathogenic microorganisms which were more resistant to antimicrobials than their planktonic counterparts. Moreover, many infections are difficult to treat with antimicrobials because of the emerging resistance of these antimicrobials. Hence *Bdellovibrio* can be used as a therapeutic agent as an alternative to antimicrobials for the treatment of oral infections.

CONCLUSION

The various in vitro studies conducted suggest that the oral application of BALO strains has the potential to rapidly decrease the number of a wide range of periodontal pathogens from the mixed oral flora and suggest a potential for *Bdellovibrio bacteriovorus* as a living antibiotic for the prevention

and treatment of periodontitis. Further in vivo studies need to be conducted in this regard.

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