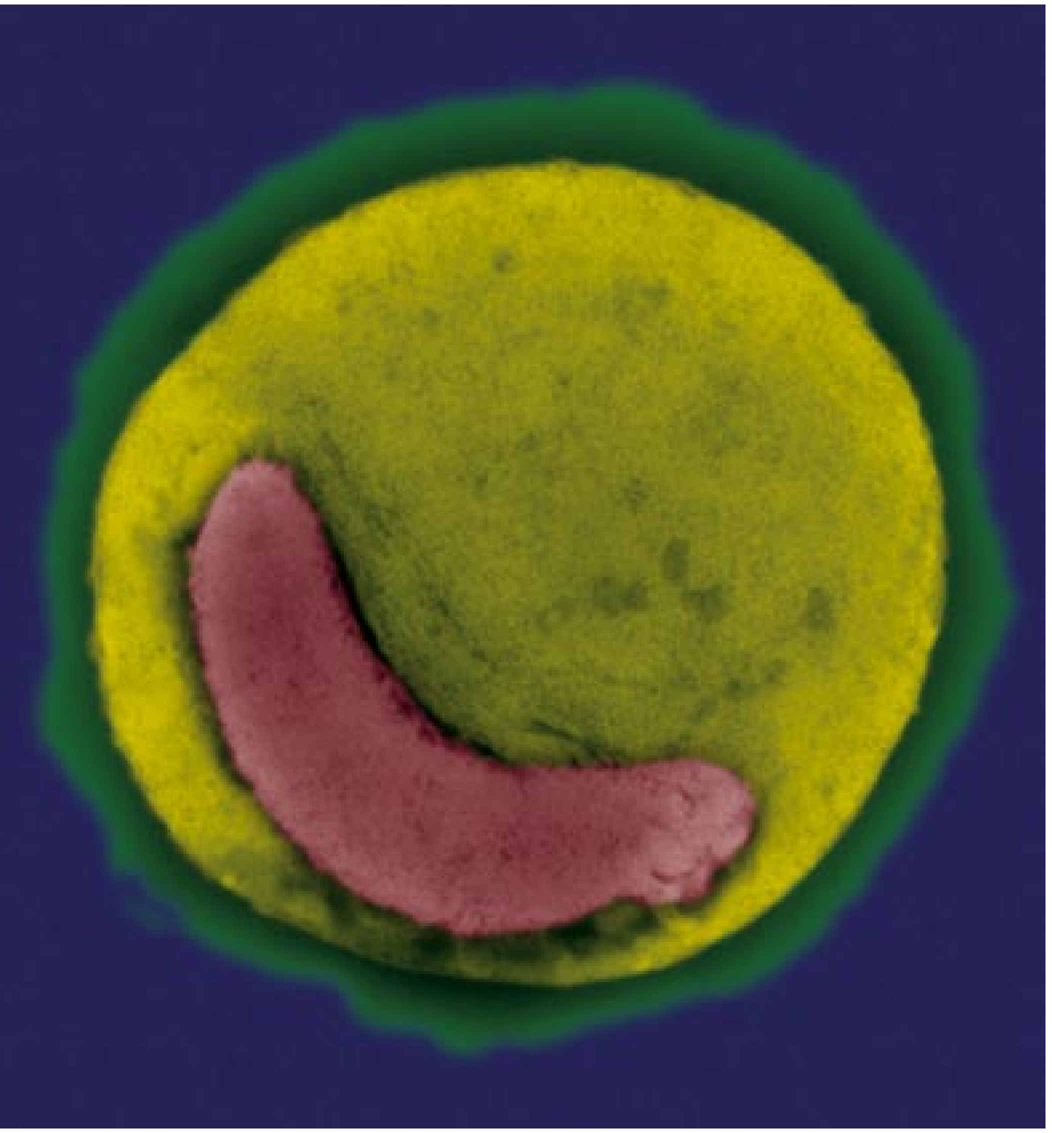
An inside job: *Bdellovibrio bacteriovorus*

Some predatory 'bugs' eat other 'bugs' from inside, as **Liz Sockett** and her research group have found.



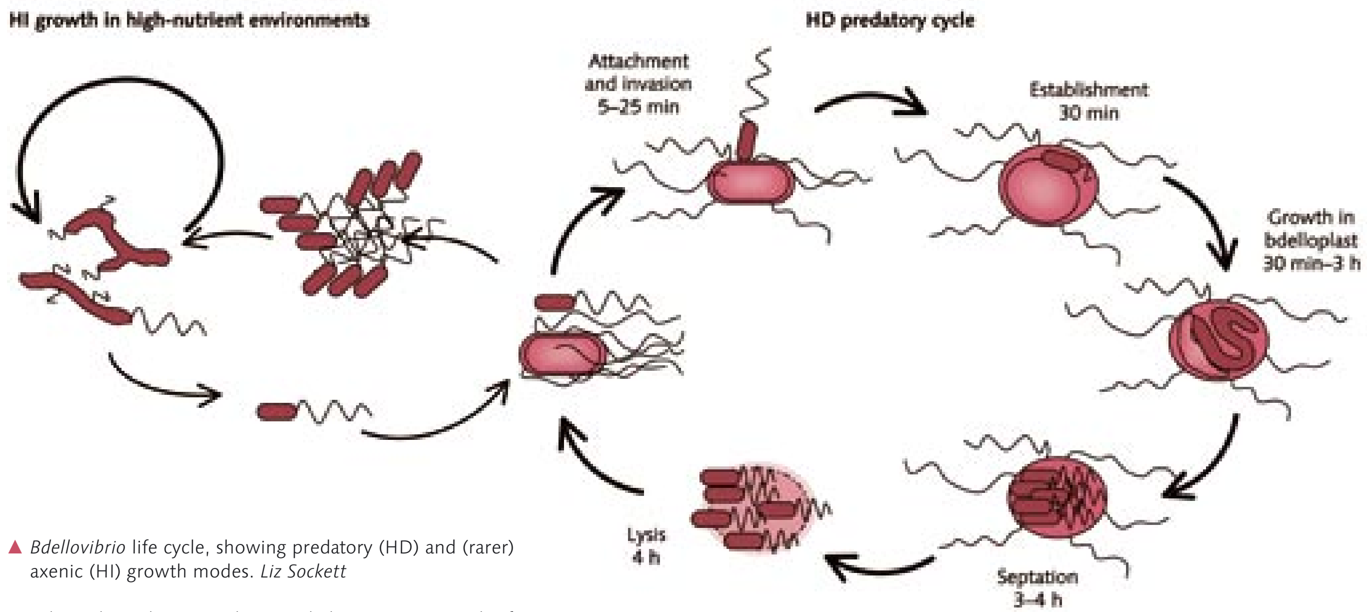
◄ False-coloured, negatively stained electron micrograph of a *B. bacteriovorus* bdelloplast. *David Milner and Laura Hobley*

Bdellovibrio are small (0.25× 1.0 µm), flagellate, motile, Gram-negative deltaproteobacteria which invade and kill other Gram-negative bacteria, entering the prey cell's periplasm, replicating within it and using the contents of that bacterium as their nutrient source. *Bdellovibrio bacteriovorus* HD100 is the sequenced strain and has a 3.8 Mb genome, indicating that although *Bdellovibrio* have evolved to invade and 'eat' other bacteria, they have not lost large numbers of genes, as is the case with truly parasitic bacteria which rely on their hosts. The interaction between *Bdellovibrio* and other bacteria can be thought of as predatory rather than parasitic as, in most cases, the *Bdellovibrio* kill the prey cell within 15 minutes of entry and do not establish a long-lived parasitic relationship within it.

In keeping with its large genome, *B. bacteriovorus* can exist in two different growth phases, as host-dependent (HD) cells that require prey for growth and division, and as host-independent (HI) cells, when growing in rich nutrient media as might be found in biofilms and sediments in nature. The balance between HD and HI growth in natural environments is not known, but HI growth is a useful tool for saving non-predatory mutants for study in the lab setting. Predatory growth seems to be a kind of evolutionary trade-off in *Bdellovibrio* where the acquisition of a large number of genes for hydrolytic enzymes, and for penetrating and resealing the prey cell, give access to an intracellular niche, bounded by the prey-cell outer membrane, where the *Bdellovibrio* can 'dine privately' on the inner contents of the prey cell without competition for nutrients from other bacteria. This contrasts with the 'eating habits' of other deltaproteobacteria such as the myxobacteria which digest prey externally and take up digested prey nutrients partly in competition with other bacteria in their surroundings.

Predatory growth

When growing predatorily, *Bdellovibrio* exhibit a biphasic lifestyle with a free-swimming attack phase and a sessile intra-periplasmic growth phase, during which they reside inside the dead prey cell, degrading it by secreting enzymes across the prey cytoplasmic membrane to digest prey macromolecules, and taking up the products for *Bdellovibrio* growth. *Bdellovibrio* seem to be mostly 'locked on' to predatory growth in dilute environments and cannot productively switch to HI growth to survive, but depend on finding prey for replication. During the free-swimming attack phase, rapid prey location, attachment and recognition are vital to the successful replication of the *Bdellovibrio*, as they typically have a half-life of about 10 hours during starvation in buffered environments. A large complement of genes for flagellar and chemotaxis systems is seen in the genome to aid movement towards prey-rich regions.



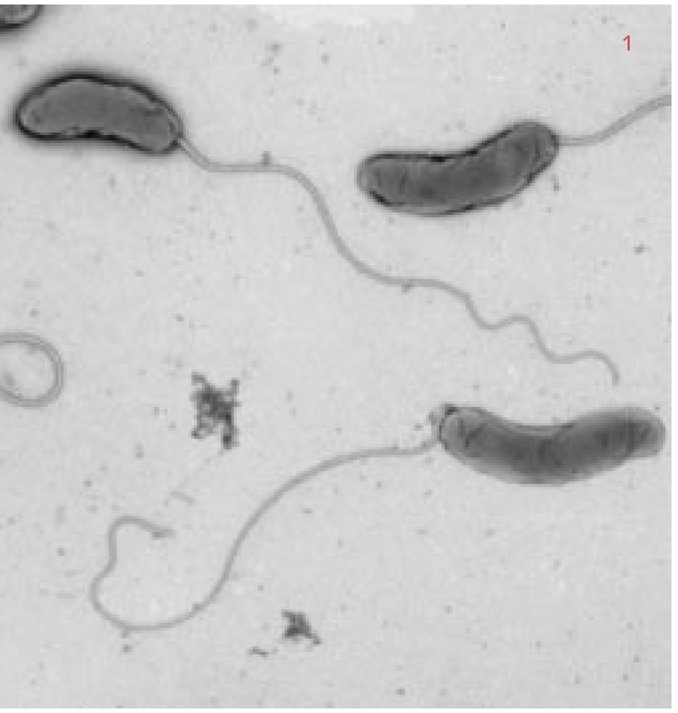
▲ *Bdellovibrio* life cycle, showing predatory (HD) and (rarer) axenic (HI) growth modes. *Liz Sockett*

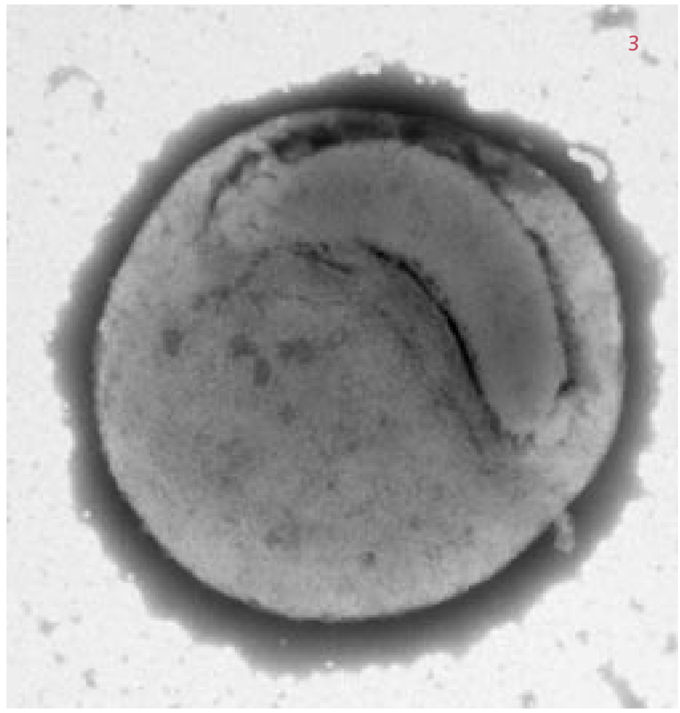
Bdellovibrio attach to many kinds of surfaces reversibly, including inorganic particles and Gram-positive cells (which are not prey as they do not have a periplasm). How they tell prey from 'junk' is unknown. There seems to be a brief recognition period, during which the *Bdellovibrio* cell identifies its prey, attachment between *Bdellovibrio* and prey becomes irreversible, and the *Bdellovibrio* begins its invasion process. A small pore is produced in the outer membrane of the prey, through which the *Bdellovibrio* bacterium seems to squeeze. Once inside, the pore is resealed, and modification of the prey's peptidoglycan causes the rounding of the prey cell and the formation of a structure called a bdelloplast. This is followed by the intraperiplasmic hydrolysis of prey, as mentioned above. The *Bdellovibrio* cell elongates, and then septates at multiple fission sites. The multiple progeny cells become flagellate, before releasing a final wave of lytic enzymes to burst from the confines of the bdelloplast and seek out more prey.

We and others are actively engaged in functional analyses of the genes within the *B. bacteriovorus* HD100 genome, studying expression patterns and defining roles for them in the *Bdellovibrio* life cycle. We have shown that, although *Bdellovibrio* swim actively using chemotaxis to find prey-rich regions, they do not use actively rotating flagella to 'bore' into prey. We have found that preventing expression of the *Bdellovibrio* PilA fibre protein of Type IV pili abolishes prey entry, showing that pili are involved in productive prey attachment and or penetration.

Where are *Bdellovibrio* found?

Bdellovibrio are found throughout nature; wherever there are suitable prey to be 'eaten', *Bdellovibrio* are usually found eating them! Soil and fresh water samples are often found to contain *Bdellovibrio*, or its closely related cousins *Bacteriolyticus* and *Peredibacter*, whilst salt-water samples often yield *Bacteriovorax* spp., another closely related cousin. *Bdellovibrio* have also been isolated from sewage, and from the gut flora of humans, horses and chickens. *Bdellovibrio* 16S rDNA sequences have been found in a variety of metagenomic studies, including those from marine sediments and even human skin. *Bdellovibrio* are found strongly associated with natural biofilms, and recent studies have shown that effective predation occurs in these naturally occurring bacterial communities.





▲ Negatively stained electron micrographs of *Bdellovibrio bacteriovorus* HD100.
(1) Free, motile, attack-phase swimming cells hoping to encounter Gram-negative bacterial prey. The cells have long sheathed flagella with an unusual wave-form caused by the presence of several different flagellin proteins making up the flagellum.
(2) Attaching to Gram-negative bacterial prey for invasion through the prey outer membrane.
(3) Living within bacterial prey forming a bdelloplast where the prey bacterium is beginning to be consumed and the *Bdellovibrio* is starting to elongate and grow prior to replication.
Andrew Fenton, David Milner and Laura Hobley

Living antibiotics!

In July 2008, the Health Protection Agency published further worrying statistics about the rise of antibiotic-resistant Gram-negative infections in the UK, charting the levels of resistance particularly in pathogenic *Escherichia coli* species. *Bdellovibrio* naturally attack and kill a wide range of pathogenic Gram-negative bacteria, including *Salmonella*, *E. coli*, *Proteus*, *Pseudomonas*, *Burkholderia*, *Serratia* and others. If applied as 'living antibiotics', *Bdellovibrio* would avoid many of the shortcomings of phage therapy which utilizes bacteriophage as a treatment against bacterial infection. *Bdellovibrio* are not known to be prey-specific; they infect a variety of Gram-negative hosts, and have no known specific host recognition sites. In contrast, bacteriophage attach to specific molecular targets, hence are only effective against a narrow range of bacteria, which can in turn become resistant by simple point mutations in genes encoding these protein targets. In addition, some phage are unable to invade cells with capsules, whereas bacterial capsules have been shown to be an ineffective barrier to predation by *Bdellovibrio*. *Bdellovibrio* have been shown to be unable to enter and infect mammalian cells, which is of great importance when considering their potential as an antibiotic treatment. This means that they could infect and kill the pathogenic bacteria causing the infection whilst not causing harm to a patient.

The potential applications of *Bdellovibrio* offer an exciting avenue for further research, and may one day form part of a new generation of antimicrobial therapeutics. In the future you may visit a doctor and be prescribed '*Bdellovibrio* therapy' to combat your infection!

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Further reading

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