Pee Wee Predator

Bdellovibrio, a rapacious bacterium, has a unique form of attack and growth

They are "parsimonious, antisocial, selfish individualists." They are "master builders" who "exercise free will," occupying a "unique and dramatic niche" in the biotic world. Microbiologist Sidney Rittenberg of the University of California at Los Angeles goes out on this anthropomorphic limb to describe his favorite bug: *Bdellovibrio bacteriovorous,* a tiny bacterial predator of other bacteria with an elaborate scheme for attack and growth. In more technical terms, *Bdellovibrio* (the B is silent) is a Gram negative, aerobic, mesophilic, chemo-organotroph — meaning it does not pick up the primary dye in the classic Gram test for bacterial taxonomy, uses oxygen, thrives in moderate temperatures and oxidizes organic materials for its source of carbon. But between the extremes of anthropomorphism and technical jargon is a fascinating example of microbial adaptability with a potential for scientific and technological exploitation.

Bdellas, as they are fondly referred to by their admirers, have a two-stage life cycle: part is spent freely swimming in microenvironments ranging from marine to soil to sewage, and part is spent within its prey where it grows and multiplies using the prey's cell contents as its food source. They were originally described in 1962 by Hienz Stolp, a West German microbiologist, as parasitic. But, because of Rittenberg's extensive studies in the past 15 years showing that a *Bdellovibrio* does not utilize another cell's metabolic machinery (like a bacterial virus) nor energy (like a parasite), most microbiologists now think of them as predators.

Under the microscope, free-living *Bdellovibrios* are extremely mobile creatures, propelled by their flagella at a rate 10 times faster than their favorite prey, *Escherichia coli.* During the attack and attachment phase, the comparatively slow and sluggish *E. coli* is struck by a *Bdellovibrio* battering ram in what appears to be a random process — three in 100 collisions result in irreversible attachment. The predator then twirls one way, the prey the other, creating a microscopic scene that Rittenberg has described as "a bevy of petite whirling dervishes with giant batons."

During this twirling process, the *Bdellovibrio* makes a hole in the outer membrane of its prey, detaches its own flagellum, and slides through to its new home, the periplasmic space between the *E. coli's* inner and outer membrane. What was once a rod-shaped *E. coli* swells into a

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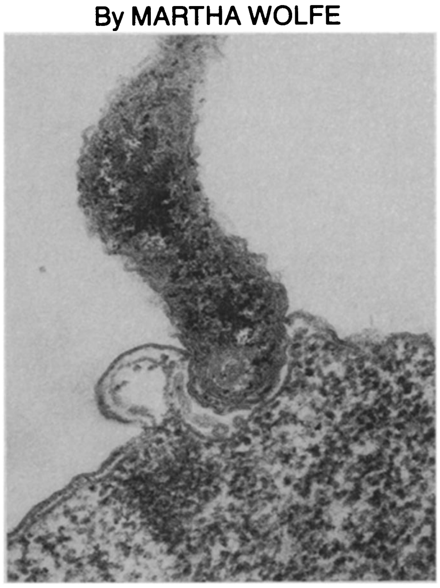
By MARTHA WOLFE



American Society for Microbiology

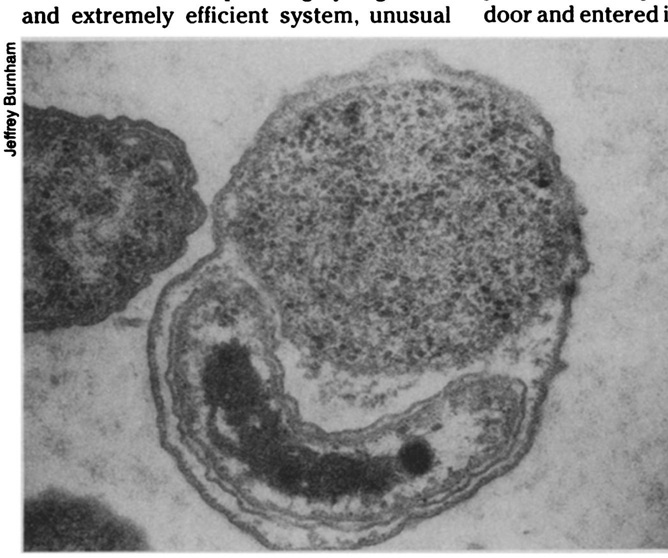
This electronmicrograph (X 55,000) shows Bdellovibrio bacteriovorus *penetrating the outer membrane of a prey* E. coli *cell.*

spherical shell and this package — the *Bdellovibrio,* its growth medium and new chamber — is called a bdelloplast, what Rittenberg describes as "a cozy isolated chamber stocked with a complete and balanced food supply." As the bdelloplast grows, the prey's protoplast (cell contents) shrinks and within two to three hours, when the food source is exhausted, the elongated *Bdellovibrio* can be seen splitting into progeny that grow new flagella, lyse the chamber walls and swim away in "search" of new prey.

The chemical correlate of the saga just described is a complex, highly regulated and extremely efficient system, unusual for a lowly prokaryote. After ramming and attaching to an *E. coli,* the *Bdellovibrio* sets about preparing its feast, but first it must enter the kitchen. Its prey is killed, presumably by the action of a bacteriocin — a chemical produced by one bacterium to kill or inhibit another. "We haven't isolated a bacteriocin yet," says Rittenberg, "but we feel sure this is what is happening." Step by step, the powerful chemical halts the prey's movement, damages its inner membrane thus allowing inorganic ions to flow in and out freely, inhibits its respiration and halts synthesis of its DNA and RNA. The physiological consequence of the poison is death, but without breakdown of the cell's contents, which the *Bdellovibrio* is preparing to consume.

After the kill, while the dervishes whirl, the *Bdellovibrio* produces at least six different enzymes that prepare an entry port in the *E. coli's* outer membrane and within 60 minutes the bdelloplast is effectively stabilized against other *Bdellovibrios* threatening to steal this one's nest. Concurrently, the attached *Bdellovibrio,* in yet another feat unique in the bacteria world, actually adds structural components to the *E. coli's* outer membrane as another stabilizing force, this time against osmotic forces shifting from the damaged inner membrane to the outer one as the bdelloplast swells. Here Rittenberg's analogy of "master builders" is apt.

"It has done an amazing thing," says Ned Ruby, a former postdoctoral fellow in Rittenberg's laboratory currently carrying out his own *Bdellovibrio* research in Los Angeles at the University of Southern California. "It has chosen a home and added to its foundation for structural purposes and for protection, then it built a door and entered into a comfortable space



Jeffrey Burnham

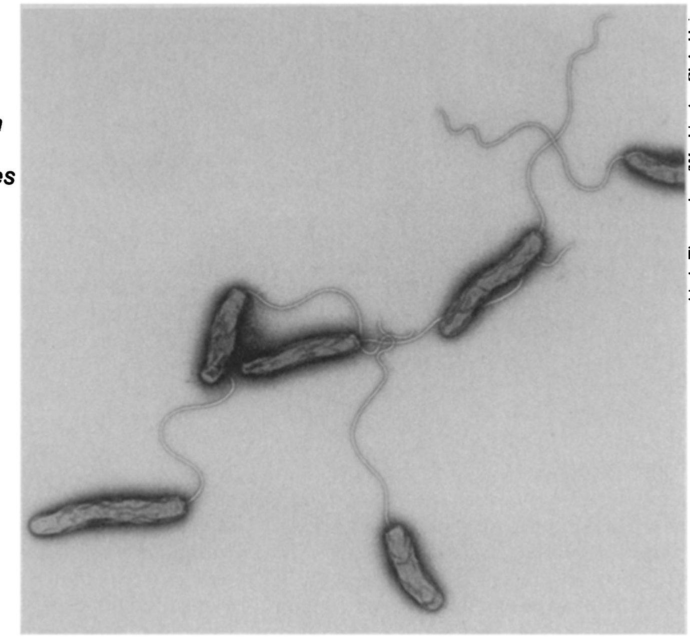
A Bdellovibrio *encapsulates itself with a prey* E. coli *cell secure against the danger of attack by other* Bdellovibrio. *It is safe from other bdellas since the chamber can only be burst from the inside out. (X 60,800)* where it can eat and grow."

Once ensconced between the inner and outer membrane, the *Bdellovibrio* carries out its own brand of genetic recycling, breaking down its food source — which comes in the form of large macromolecules—into useful monomers. "As far as we can tell," says Dana Diedrich at Texas Tech in Lubbock, another *Bdellovibrio* enthusiast, "the *Bdellovibrio* doesn't just blow open the cytoplasm and gobble up the contents. Rather, it has made the inner membrane permeable to large molecules that couldn't get out before, so it consumes them as they slowly filter into the periplasmic space." Using endo- and exonucleases — endonucleases make internal cuts in DNA and RNA molecules and exonucleases cut from either end — the *Bdellovibrio* digests the prey's genetic machinery into single nucleotides that it can then incorporate into its own. "The elegance of this process," writes Rittenberg, "is in the very small loss of DNA precursors in the growth process."

When the *E. coli's* cell contents are consumed, the *Bdellovibrio* prepares to move out of its "cozy" house. The now-elongated *Bdellovibrio* fragments into a number of progeny corresponding to the size of the cell just eaten — anywhere from four to hundreds. The new ones acquire flagella and then release another enzyme—which only works from the inside out — to lyse their cocoon and free themselves.

Knowing the *Bdellovibrio's* behavioral and chemical habits, it becomes easy to extrapolate on possible exploitations. "They're so promiscuous with what they eat," says Diedrich. "It's only natural to wonder if they exist as biological control factors." In fact, little is known about the ecology of *Bdellovibrios,* their numbers and their role in the communities where they are found. Do they, for instance, work as natural population controllers, keeping the numbers of other microbes in check? "We met with little success trying to use them to attack blue-green algae," says Jeffrey Burnham of the Medical College of Ohio in Toledo. His group was trying to exploit *Bdellovibrios* to control algal blooms in fresh water environments, where the algae cause severe oxygen depletion and subsequent fish kills. "I've not given up on bdellas. Someday somebody will find them useful," he says.

Their brightest future may be as models for other biological systems. A favorite organism for geneticists, working on ways to encourage microorganisms to mass produce substances like insulin and interferon, has been *E. coli* (SN: 1/26/80, p. 52). But *E. coli's* repertoire does not include a delivery system — most *E. coli* that have been genetically engineered to produce these economically important substances refuse to spit them out, so yields can be very low. "I believe *Bdellovibrio* can secrete substances," says Diedrich. "They make huge amounts of proteases and other enzymes. If we could find out how they do that, maybe we could genetically engineer *E. coli* to do the same."

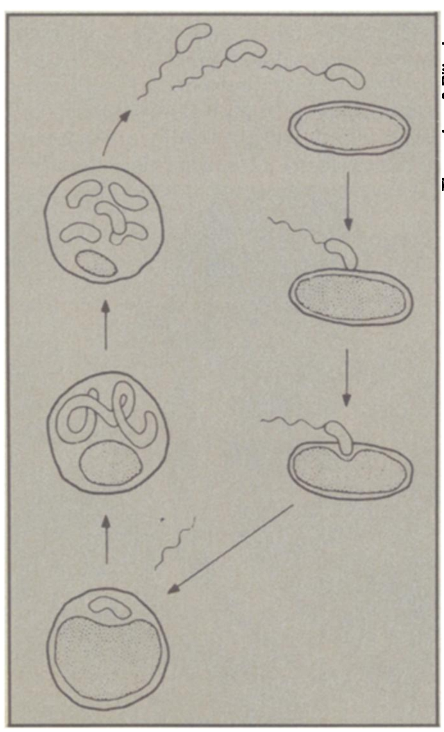


Linda Thomashow/Washington State Univ.

Bdellovibrio bacteriovorus *cells shortly after release from their growth chamber, an* E. coli *bacterium. The mobile creatures are magnified here 9,900 times.*

About genetically engineering *Bdellovibrios,* Diedrich says, "I can imagine engineering *Bdellovibrio* to perform a certain task." Not everyone agrees. Says Ruby, "It is more likely that we will use genetic engineering to understand *Bdellovibrio* rather than the other way around."

One step toward the more abstract in biological models is the use of *Bdellovibrio* as a general example of membrane and protein structure-function relationships. "We know that the *Bdellovibrio* steals entire proteins from their prey and incorporates these proteins into their own membrane," says Diedrich. "If we know what it is in the protein's surroundings that allow it to be mobilized, we could be closer to understanding membrane systems and the role of proteins within them."



Thomashow & Rittenberg

During the three to four hour life cycle a predatory Bdellovibrio *attacks, enters and grows within its prey, then multiplies and bursts out to repeat the process elsewhere.*

On another important front in biological systems, Ruby comments, "*Bdellovibrios* are one of the few bacteria which can differentiate (change functions according to the stage of its life cycle). "I would like to know more about how it does this, what triggers it to switch from attack to growth and back to attack phases. We could then better understand how eukaryotes control differentiation, which is applicable to cancer cells because cancer cells lack the ability to differentiate."

Diedrich and Ruby represent the second generation of microbiologists studying *Bdellovibrio.* Ruby studied under Rittenberg, who is nearing retirement, and Diedrich was under S.M. Conti at the University of Virginia in Charlottesville. They are members of a small group, studying an esoteric subject in the broad field of microbiology, but both men have a common reason for pursuing it: their love for this fascinating bug. "No matter what kind of high-brow biochemical and physiological piddling you profess to do, there's nothing like sitting with a microscope and watching these bugs," says Diedrich. "They afford me a novel approach to the very competitive field of physiology of microorganisms, but I also love watching them."

"I must confess to not being a very pragmatic scientist," says Ruby. "We could spin some tales of how useful they could be, but to me they are fascinating because they are so very good at what they do." Rittenberg, who both younger men agree is the master, says, "*Bdellovibrio's* most vast potential is for understanding the breadth of the adaptability of life." □