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Being Bad is Best for Bacteria

For a pathogen trying to survive in the microbial jungle, being baddest is best.

That conclusion is based on the first experiments investigating how natural selection influences the transmission of infectious disease. The outcome of those experiments defies old assumptions that pathogens evolve to become less infectious, said Howard Hughes Medical Institute international research scholar Brett Finlay, who is at the University of British Columbia, Vancouver.

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— **B. Brett Finlay**

The research, which is published in the April 19, 2007, online issue of *Current Biology*, found that genes that enhance the virulence of bacteria are clearly favored for evolutionary survival. Numerous species of disease-causing bacteria share these virulence factors and use them to manufacture a common weapons system. The study offers a new model for scientists to study transmission of any infectious disease, and, Finlay said, could help identify new therapeutic targets for blocking the spread of pathogens.

What we do in this paper is study something never studied before, which is how transmission evolves to allow bacteria to be passed from host to host, Finlay said.

Over the past decade, Finlay's laboratory has uncovered an awe-inspiring molecular complex that many virulent bacteria, including the bad actors among *E. coli*, employ to infect mammalian cells. Bacteria use this complex, called a type III secretion system, to command a host cell to build a portal on its surface and then deliver an assault of infectious proteins into host cells through a tube-like connector. A group of bacterial genes is behind the virulent weaponry.

Bacteria that cause bubonic plague, *Pseudomonas aeruginosa* infection, typhoid fever, and bacterial dysentery all possess similar type III secretion systems. These bacteria can share their virulence genes, passing them between individuals in a process known as horizontal gene transfer, which has aided and abetted microbial evolution from the beginning.

According to Finlay, the phenomenon can be compared to file sharing. Bacteria have their own Internet, he said. They can download each other's genetic sequences. Scientists can easily determine which genes are most popular by looking at the number of microbial species that have downloaded them. Genes common to multiple species are considered important for long-term survival. Genes that allow infectious microbes to spread to as many hosts as possible are favored.

What makes a pathogenic strain of *E. coli* infective are virulence factors, which are mobile genetic elements that allow DNA to be transmitted from organism to organism, Finlay said. We knew from our previous work that all these virulence factors are moving around. What this work shows is there is a selection pressure involved, which is the ability to transmit disease from host to host. Previously, microbiologists looking at infectious disease have focused on the disease, but we wanted to look at how the disease influences its infectiousness. That is, the old approach centered around how these swappable genes contribute to disease in one host. Now we know that the advantage they give to nasty bacteria in this first host contributes to getting to the next host - so being bad is good.

Finlay and his team, including lead authors Mark Wickham and Nat Brown, created a model of natural selection that demonstrates how type III secretion system genes contribute to an organism's fitness. For their experiments, they used a bacteria known as *Citrobacter rodentium*, which infects mice using the same virulence factors that *E. coli* (such as enteropathogenic and enterohemorrhagic *E. coli*) use to cause disease in humans. Using a strain of mouse that dies during *C. rodentium* infection, the team exposed groups of mice either to the normal pathogen or to versions in which different type III secretion genes had been eliminated. This produced various degrees of pathogenicity, ranging from mild to fatal.

The researchers conducted a series of experiments in which mice were infected, then exposed to uninfected mice, which were in turn exposed to more uninfected mice. We were not interested in any dead-ends - the infections acquired needed to be able to be spread further, Finlay explained.

The scientists analyzed the patterns of disease transmission and found that strains of bacteria with the greatest damage to their virulence genes were slowest at spreading from one host to another. The strain of pathogen with all of its virulence genes intact spread the fastest. Strains of pathogen lacking the ability to inject any proteins into the host were completely unable to spread between hosts, suggesting that these swapped virulence genes are essential for spread.

In the ultimate test, mice were fed broth containing an equal mixture of normal bacteria and bacteria containing defective weaponry genes. After 72 hours, these mice were housed with uninfected mice long enough for a moderately defective strain of bacteria to spread. The newly infected mice were housed with another group of uninfected mice for 24 hours. Then that group of newly infected mice was exposed to yet another fresh group. The process was repeated out to 10 iterations or stopped two generations after the defective bacteria disappeared from the infection. By examining how many cycles of infection it took for the normal bacteria to take over, out-competing the bacteria whose virulence factors had been damaged, the researchers could determine the relative contribution of each virulence gene to the survival of the species.

The results showed that the normal pathogen always out-performed defective strains, which disappeared from the infection with a speed determined by the severity of their virulence defects. Finlay said the results show that each of the genes involved in the infectious weaponry offers a clear survival advantage to the bacteria.

The key point is we can now study disease transmission, which we haven't really done before. The methods could be used to model transmission of any infectious agent and that gives us a new capacity to understand disease, said Finlay. But this also could be important for treatment. Now that we can study transmission, we are looking for other genes that are involved that we currently know nothing about. All of these genes might become targets for blocking the transmission of infectious diseases.