Once thought unstoppable, bacterial superweapon falters with too many targets

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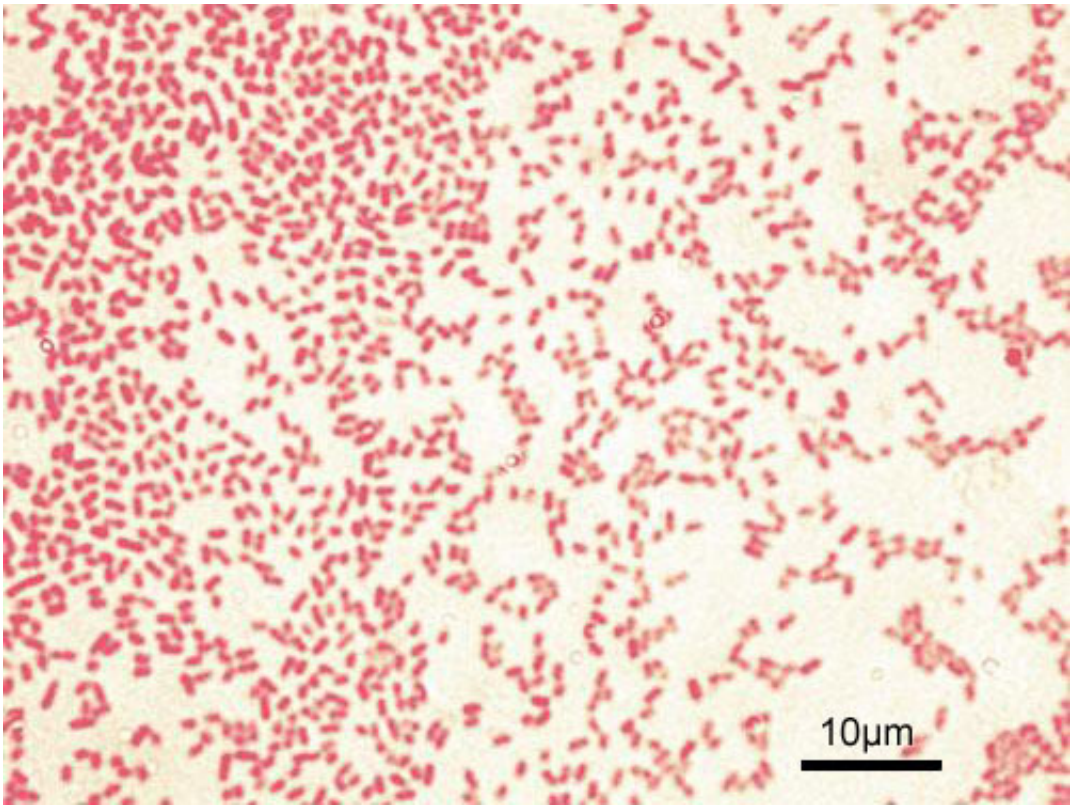

Gram-stained P. aeruginosa bacteria (pink-red rods) Credit: Wikipedia

In 2006, scientists discovered that some of the world's most common disease-causing bacteria wield a uniquely powerful weapon that can kill targeted cells—both other bacterial cells and membrane-enclosed eukaryotic cells—by injecting them with toxic proteins.

The type VI secretion (T6S) system is a devastating quick-kill mechanism that has been found to exist in nearly a quarter of gram-negative bacteria, including *Vibrio cholerae*—the infectious agent that causes cholera—and *Pseudomonas aeruginosa*, a versatile pathogen that can cause sepsis and organ failure.

New research from Princeton University and the University of Basel in Switzerland has revealed that T6S, once thought to be a microbial superweapon, can be thwarted if groups of targets are large enough when the assault begins. Although organisms on the outside of the cluster will perish, the protected interior cells can multiply quickly enough to replenish the group's numbers from the inside—even to the point where the number of targets spawning exceeds the number dying—and the attacking bacteria cannot take over.

Reported in the journal *PLOS Computational Biology*, the findings—which combined computer simulations with observations of living bacteria—could provide insight into how cells withstand powerful aggressors, which scientists could use to develop treatments against pathogens.

"Having T6S is not a 'Terminator' weapon, which is what it looked like initially and what a lot of people thought," explained first author David Borenstein, a postdoctoral research associate in Princeton's Lewis-Sigler Institute for Integrative Genomics. "We now think that the reason more bacteria don't have it is because it's not necessarily a good weapon."

The researchers suggest that T6S may be only an occasional weapon used to eliminate specific targets under certain circumstances, which would help prevent the accidental killing of beneficial bacteria. The secretion system also is highly energy-intensive, Borenstein said. Thus, bacteria relying primarily on T6S would be laboring to constantly produce toxins.

"There seem to be certain circumstances in which T6S is useful," Borenstein said. "It's not just turned on and fired willy-nilly in every direction. It's definitely something bacteria are choosing to use."

In a new twist, the researchers found that the T6S system also has a potential role as a defensive weapon when cells with T6S attack other cells that also have it. While immune to their own secretion systems, the cells can kill each other. A computer simulation showed that when T6S-equipped bacteria attacked others, the organisms with the majority population won out.

"If bacteria have T6S and an established population, it will be much easier for them to defend against an invading microbe, even if the attackers have it, too," Borenstein said. "The T6S system is a way to have a standby defense without producing defense toxins all the time and without inadvertently killing bacteria that might be beneficial in the meantime."

The fusion of simulations and laboratory work are a notable feature of this work that could be used to better explore other biological systems and interactions, said Jeff Gore, an associate professor of physics at the Massachusetts Institute of Technology. The work illustrates that unique and potentially important ideas can spring from the interchange of computer modeling and experimentation, said Gore, who is familiar with the work but was not involved in it.

"This paper represents a wonderful example of combining biologically motivated modeling with laboratory experiments," Gore said. "This is a powerful mode of inquiry that in my opinion could be used fruitfully to elucidate many other biological systems. In particular, more modeling should be motivated by surprising experimental results, and more quantitative experiments should be motivated by surprising theoretical predictions."

Borenstein and co-author Ned Wingreen, Princeton's Howard A. Prior Professor of the Life Sciences and professor of molecular biology and the Lewis-Sigler Institute for Integrative Genomics, first simulated the assault by cells with T6S on cells vulnerable to the secretion system. The target cells' resilience was surprising, Borenstein said.

"The phenomenon we saw is similar to a herd of animals that cluster around each other when predators attack—the individuals on the outside are vulnerable, but the interior of the community is protected," Borenstein said. "But in this case, the prey animals on the inside rapidly reproduced during the assault. Once there were enough, it didn't matter how many predators there were anymore—they couldn't win."

Co-authors Marek Basler, a professor of biology, and graduate student Peter Ringel, both at the University of Basel, tested the simulations in the laboratory on live bacteria by pitting *V. cholerae* against the bacteria *Escherichia coli*, which are vulnerable to T6S, and found the same results. A 2013 paper published in the journal *Cell* on which Basler was the first author inspired the current research.

The simulations allowed the researchers to not only build upon their previous laboratory work, but also realize theories that would be difficult to physically carry out, Basler said.

"Even before this collaboration, my group and others in the field made certain observations that we were explaining only intuitively," Basler said. "One beauty of these simulations is that one can vary parameters that are not so easy to vary experimentally, such as the killing rate or growth rate, and learn what would happen in a competition of bacteria strains under completely different conditions.

"In many cases, the way we set up our competition assays in the lab is artificial; in nature you hardly see exponentially growing bacterial

communities," he said. "But here we showed that the prey cells can win by outgrowing the competition even though they are constantly getting killed. I believe that in the future, we will discover more strategies about how prey cells deal with aggressors."

Wingreen initiated the project after reading Basler's 2013 paper. That study showed that when *P. aeruginosa* attacked *V. cholerae* and the bacteria *Acinetobacter baylyi*—which also has the T6S system—they only resorted to using T6S when they detected that their targets also were using it. Wingreen began thinking that perhaps the use of T6S is selective and that there are costs and benefits bacteria consider, he said. He approached Borenstein, a software engineer, who had designed a simulation program called Nanoverse that predicts the outcomes of biological processes.

"We quickly realized that the spatial structure of the competing strains are crucial to the outcome—there is a critical domain size above which sensitive colonies will survive," Wingreen said. "Real biology is always more complicated than our models, so to confirm that this simple idea actually held up in a real system, we initiated an experimental collaboration. [Basler and Ringel's] observations confirmed our main prediction—large colonies can survive an attack while small ones perish."

The dynamic the researchers uncovered also could apply to other natural scenarios in which a vulnerable organism faces a powerful assailant, such as coral reefs struggling to resist algae, Wingreen said. Such an organism's resilience might depend on strengthening its pre-assault population and ensuring that it can maintain steady regeneration during the onslaught.

Indeed, Gore said, the researchers show that considerations such as spatial structure can supersede principles otherwise presumed to be true.

"Given that toxin-producing strains can kill toxin-sensitive strains, it is natural to assume that toxin production will always spread throughout a population," Gore said. "However, these researchers have demonstrated that the fate of toxin-production in a population depends critically on the size of the domains that each of these strains occupies.

"The microbial world is full of examples of cells interacting in rich ways, either competitively or cooperatively," he said. "This work highlights that the range of that interaction can be very important."

The paper, "Established microbial colonies can survive type VI secretion assault," was published by *PLOS Computational Biology*.

More information: David Bruce Borenstein et al. Established Microbial Colonies Can Survive Type VI Secretion Assault, *PLOS Computational Biology* (2015). DOI: 10.1371/journal.pcbi.1004520

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