Bacteria show surprising number of genetic paths to survival

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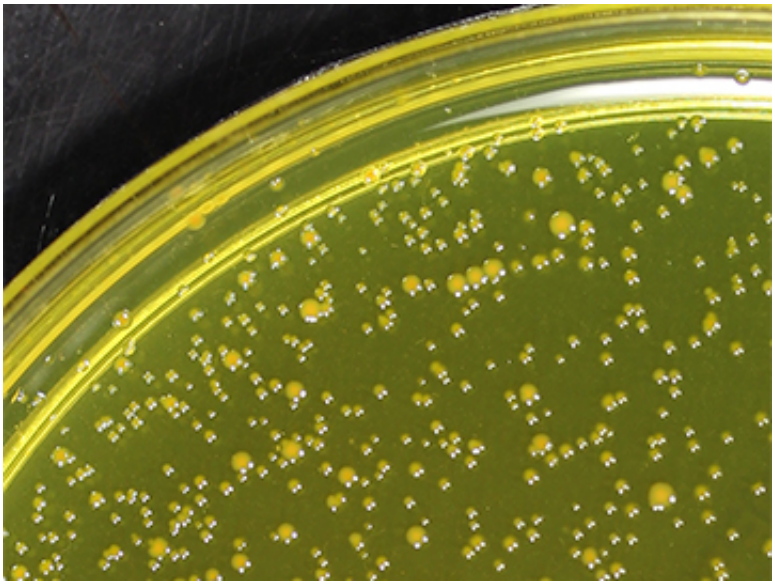

There may be much more genetic diversity within a single species of bacteria in an infected person than previously assumed. Credit: Tami Lieberman

(Phys.org) —A boy with cystic fibrosis develops a chronic and potentially deadly Burkholderia dolosa infection in his lungs. Varieties of genetic mutations allow some strains of the bacteria to survive the dual assaults from his immune system and antibiotics, while others perish. Eventually, the strongest mutant dominates the B. dolosa colony. Right?

Maybe not, say the authors of a new study. Examining sputum samples

from infected patients, they found that dozens of different kinds of B. dolosa may coexist in that boy's lungs. Instead of a majority genome representing the "best" way to survive in their combative environment, groups of B. dolosa exhibit different genetic variations that have helped them adapt in different ways.

"We found that when a pathogen like B. dolosa infects us, it diversifies. Many cells discover ways to survive, and these successful mutants coexist," said Roy Kishony, professor of systems biology at Harvard Medical School and senior author of the study. "This suggests we can reject the model in which a single mutant starts to grow better than everyone else and takes over."

Published online Dec. 8 in *Nature Genetics*, the findings not only reveal new insights into human infection, bacterial evolution and B. dolosa biology, they also provide a warning about possible shortfalls in current methods of testing and treatment.

"The study may change how we think about infections," said co-senior author Gregory Priebe, HMS associate professor of anesthesia at Boston Children's Hospital and an infectious diseases researcher at Brigham and Women's Hospital. "We found that these bacteria have incredible diversity and an amazing power to evolve and adapt to different environmental stressors."

Peaceful co-existence

The study grew out of a puzzling discovery most of the current team—including Kishony, Priebe, co-senior author Alexander McAdam and first author Tami Lieberman—made two years ago while they were examining bacteria from cystic fibrosis patients who had acquired chronic B. dolosa infections during an outbreak in a single center in Boston in the late 1990s/early 2000s.

They expected B. dolosa cells from later samples to look like genetic descendants of those in earlier samples. Instead, the family tree was all mixed up.

"Something was fishy," said Kishony. He suspected the answer to the riddle lay in the assumptions that influence the way bacteria are typically cultured.

A tube of sputum taken from a patient contains thousands or even millions of bacterial cells from the infection. Clinical labs select a small number of colonies, each grown from an individual cell in the patient's sample, and perform identification and antibiotic susceptibility testing on them. If all or even most of the B. dolosa cells in the original sample had the same genome, then those few colonies are representative of the patient's bacterial population. But if there were a dozen or more B. dolosa variants in the mix, then doctors and researchers see only a fraction of the picture, like the proverb about blind men feeling different parts of an elephant.

"We needed to find out how one B. dolosa cell differs from other B. dolosa cells in the same sample," said Kishony.

For this study, the researchers acquired a new set of patient samples to measure the diversity in each one.

The team gathered sputum samples from five cystic fibrosis patients who have harbored decade-long B. dolosa infections from the Boston outbreak. Instead of studying single isolates, the researchers cultured many cells from each patient and conducted two kinds of genetic sequencing (colony resequencing and deep population sequencing) to reveal B. dolosa's genetic diversity.

"There was so much more diversity than we expected," said Lieberman,

who is a doctoral student in Kishony's lab. "And the diversity appeared to have existed for many years."

In 29 isolates cultured from a single sputum sample from one patient, Lieberman found six distinct B. dolosa lineages and 188 different mutations. Out of the 406 possible isolate pairs, an average of 26 mutations distinguished any given pair, and only 1 pair was identical. Only 10 mutations were shared by every isolate.

"Because the different lineages coexist for a long time, we can read the record of past selective pressures within a particular individual. We see what the bacteria adapted to," said Kishony. "We found we can get more information by looking deeply and carefully at a single snapshot than we could from following an infection at different times. We can learn a lot about the past without needing old samples."

For instance, coexisting in one patient alone the team counted three distinct mutations in a B. dolosa gene that makes a penicillin-binding protein, suggesting that the bacteria often evolved to survive treatment with drugs related to penicillin. Meanwhile, other bacteria had mutations in oxygen-sensing and iron-scavenging genes that told different stories about the challenges they faced in a patient's lungs.

Future implications

In addition to illuminating basic biology, the study suggests we may need to change how bacterial cultures are performed, how treatments are selected for bacterial infections and how epidemiologists piece together bacterial transmission from person to person.

If the findings in B. dolosa also apply to other pathogens, "We may need to rethink the practice of studying single bacteria colonies from a patient," said Lieberman.

"The great diversity of B. dolosa in these samples suggests that clinical laboratories might need to analyze a broad representation of a bacterial population in a patient to understand his or her longstanding infection and select the appropriate antibiotics for treatment," said McAdam, HMS associate professor of pathology at Boston Children's.

Since the team studied only one species of bacteria and that species is mostly contained within a small group of patients, "it's open to be seen how general our findings are to other pathogens, both in long-term and short-term infections," said Kishony. He hopes researchers will apply his team's approach to other bacteria to find out.

"The hope is that whole-genome sequencing of bacteria will shed light on characteristics that have not been routinely investigated in clinical microbiology, such as the specific mechanisms by which the bacteria survive in the lung and cause damage to the host tissue," said McAdam.

"Personalized medicine currently looks at the human genome to tailor treatment," Kishony added. "We may be headed in a direction where we want to look at variations in the pathogen's genome in different people."

"If we know what the bacteria evolve against in a person's body, then perhaps one day we can strengthen the specific challenges they face until they can't survive anymore," speculated Lieberman. "We may eventually turn to new treatment approaches, including bacterial species-specific approaches, rather than the sort of large hammers we're currently using that disrupt our microbiomes and cause collateral antibiotic resistance."

B. dolosa and beyond

To explore how B. dolosa's genetic diversity may relate to the physical niches in which it lives in the body—"whether one genotype lives in the right lobe of the lung and another in the left, or whether instead they

coexist throughout the lungs," as Lieberman put it—members of Kishony's lab are examining tissue samples from 30 different sites in the lung.

In addition, the team intends to investigate whether genetic variants in B. dolosa can be correlated to different physical manifestations of disease.

Kishony and colleagues have begun follow-up studies to determine how generalizable their findings are. For example, they are looking at the pathogen Mycobacterium tuberculosis in collaboration with Ted Cohen, associate professor of epidemiology at Harvard School of Public Health, and others.

Priebe and Kishony are also studying whether the bacterium Pseudomonas aeruginosa shows comparable genetic diversity to B. dolosa during acute infections of only a few days or weeks in ICU patients.

"Do bacteria adapt in such a short time? Does that adaptation also lead to diversity?" asked Kishony.

More information: Genetic variation of a bacterial pathogen within individuals with cystic fibrosis provides a record of selective pressures." Tami D Lieberman, Kelly B Flett, Idan Yelin, Thomas R Martin, Alexander J McAdam, Gregory P Priebe, Roy Kishony. *Nature Genetics* (2013) DOI: 10.1038/ng.2848

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