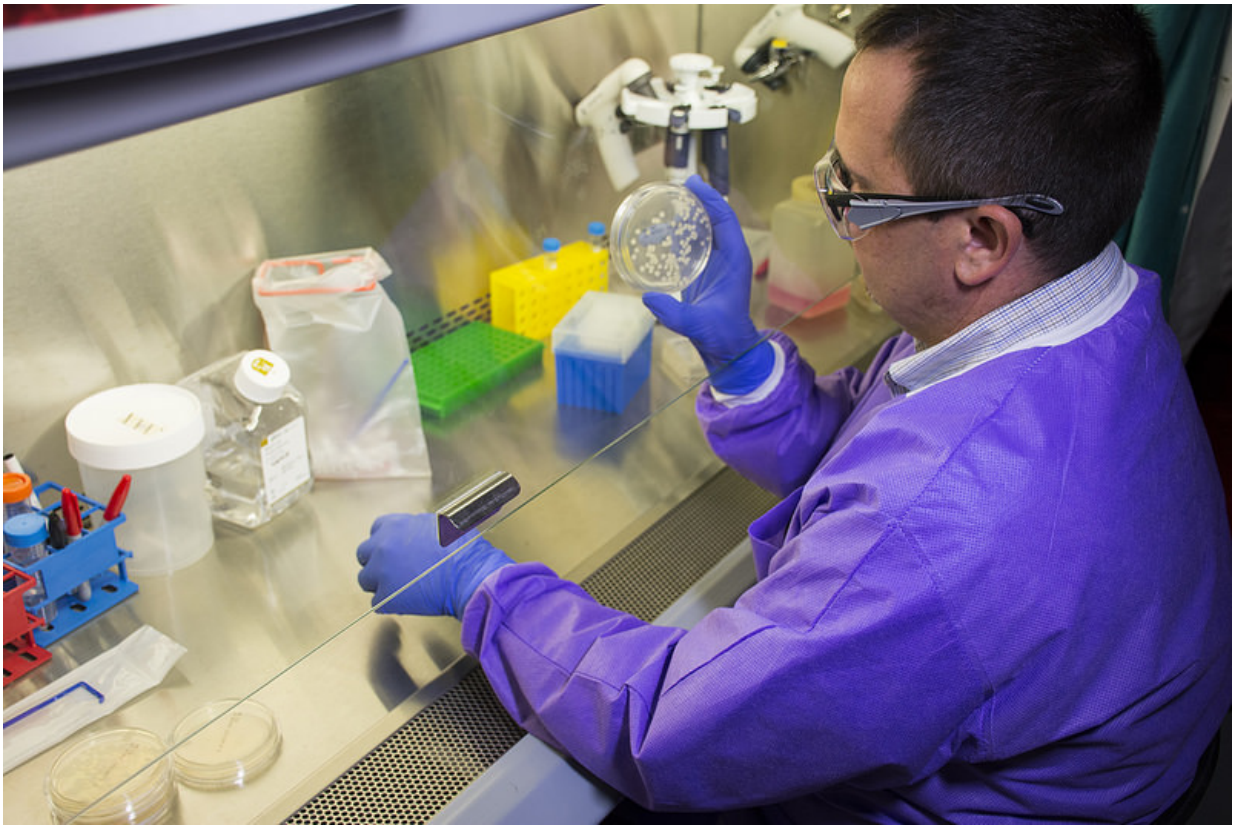
How anthrax spores grow in cultured human tissues

June 23 2015, by Greg Koller



PNNL microbiologist Josh Powell looks at anthrax spores, which have developed into bacteria over the course of 12 hours. At low doses, researchers found growth of spores is lower in human lung cells than rabbits.

Cultured human lung cells infected with a benign version of anthrax

spores have yielded insights into how anthrax grows and spreads in exposed people. The study, published in the *Journal of Applied Microbiology*, will help provide credible data for human health related to anthrax exposure and help officials better understand risks related to a potential anthrax attack.

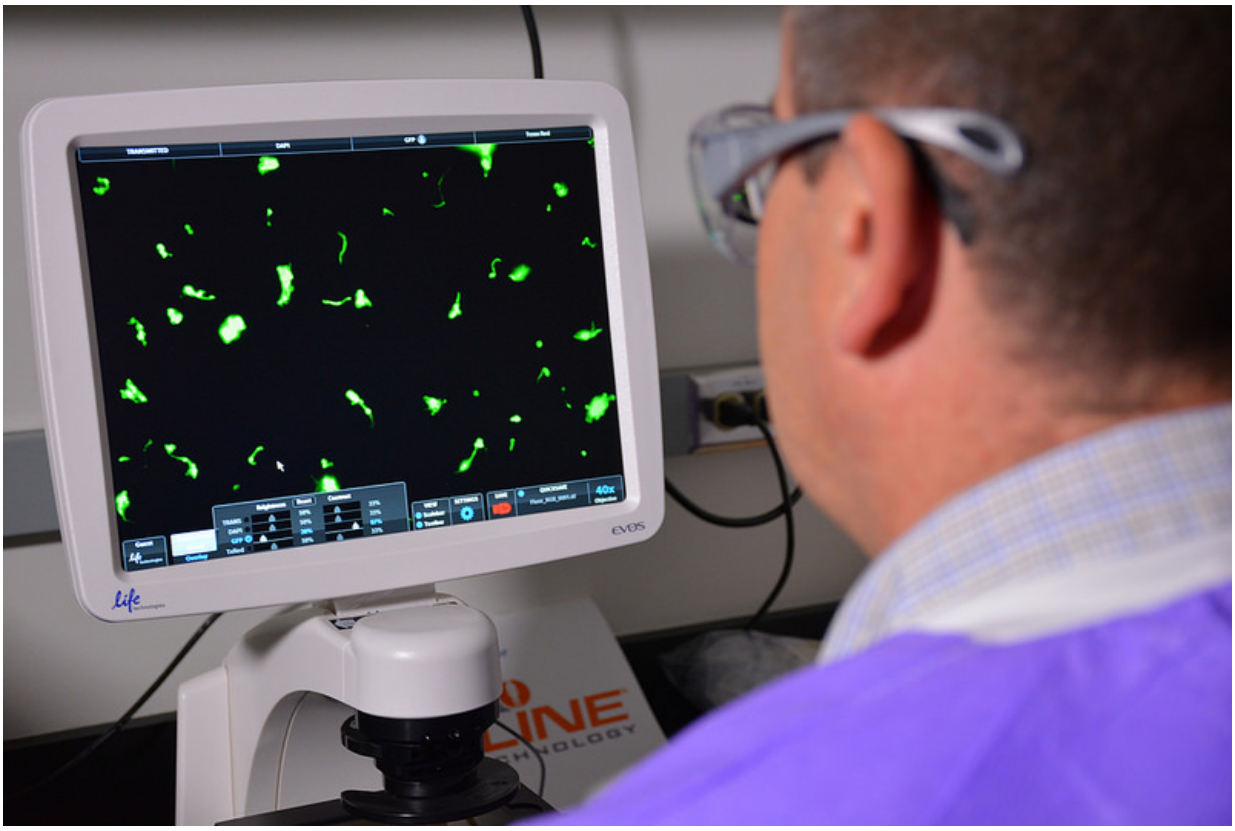
The study also defined for the first time where the spores germinate and shows that the type of cell lines and methods of culturing affect the growth rates.

"What we're learning will help inform the National Biological Threat Risk Assessment—a computer tool being developed by the Department of Homeland Security," said Tim Straub, a chemical and biological scientist at the Department of Energy's Pacific Northwest National Laboratory. "There is little data to estimate or predict the average number of spores needed to infect someone. By better understanding exposure thresholds, the ultimate goal is to be able to predict outcomes from terrorist incidents involving Bacillus anthracis."

There are decades of data characterizing anthrax exposure in rabbits, but there is limited understanding of how this data extrapolates to humans. When researchers delved into this, working from cultured normal lung cells from each species, they found that, at low doses, the proliferation of anthrax spores is lower in human lung cells.

It's too early to say what that means for human health, but the study's methods and results may resolve a long-standing debate on the pathogen's propagation. Researchers showed that anthrax spores germinate in the lungs before making their way to the bloodstream. That has been a point of debate in the research community, with some speculating that spores, which are invisible to the naked eye, must first enter the blood stream and then grow into bacteria that can cause damage and death.

Knowing the precise location and pathway of spore germination and understanding that the bacteria begin producing toxins that damage tissue directly in the lungs may eventually impact treatment options. The finding also likely indicates added susceptibility in individuals who already have lung issues, such as smokers or those with asthma.



PNNL microbiologist Josh Powell views anthrax bacteria and spores magnified 400 times. His research shows anthrax grows differently in rabbit and human lung cells and between cancerous cell lines and normal cultured cells from each species.

Making conditions real

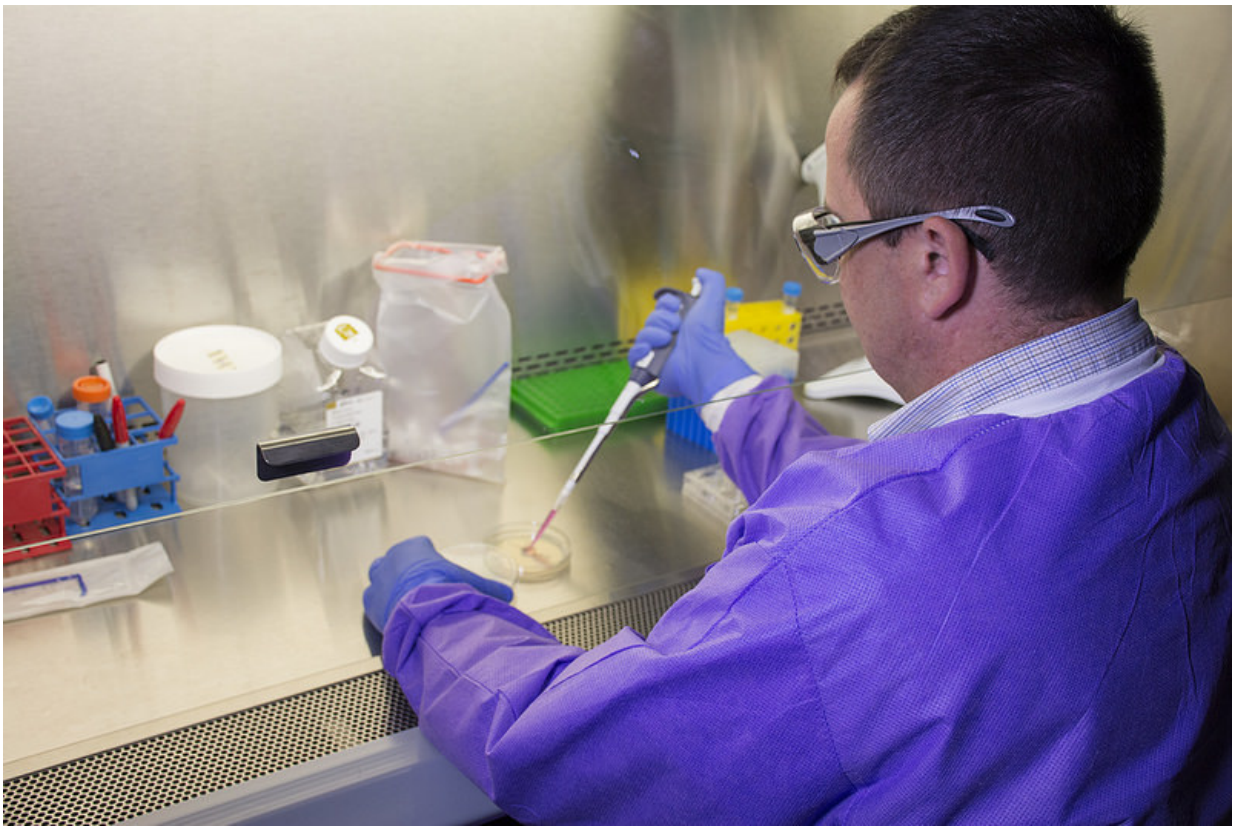
Most of what researchers know about anthrax comes from studying cancerous lung cells of both humans and rabbits because they are easy to grow in a lab. But cancer cells are very different from normal cells, which are referred to as primary cells.

For this study, PNNL researchers wanted to see if normal cells reacted differently. So, they carefully cultured primary rabbit lung cells on special inserts in petri dishes, coaxing them to form small pieces of 3-D lung tissue about the size of a quarter.

"The cells are fed with nutrients from below and we trick the top layer of cells into thinking they are at the air/liquid interface as they would be in a living lung," said Josh Powell, a microbiologist at PNNL.

Researchers observed the top layer of cells producing sticky mucus, which traps the anthrax spores. This did not occur with cells completely submerged in the growth medium where the spores just float on top. This suggests that this mucus facilitates germination of the spores into bacteria.

"Byproducts secreted in the mucus by lung cells, in reaction to the anthrax, cause the spore to proliferate very quickly," said Powell. "We don't know what those byproducts are yet, but this is the first time it's been shown that growth rate is impacted by these byproducts secreted by the lungs."

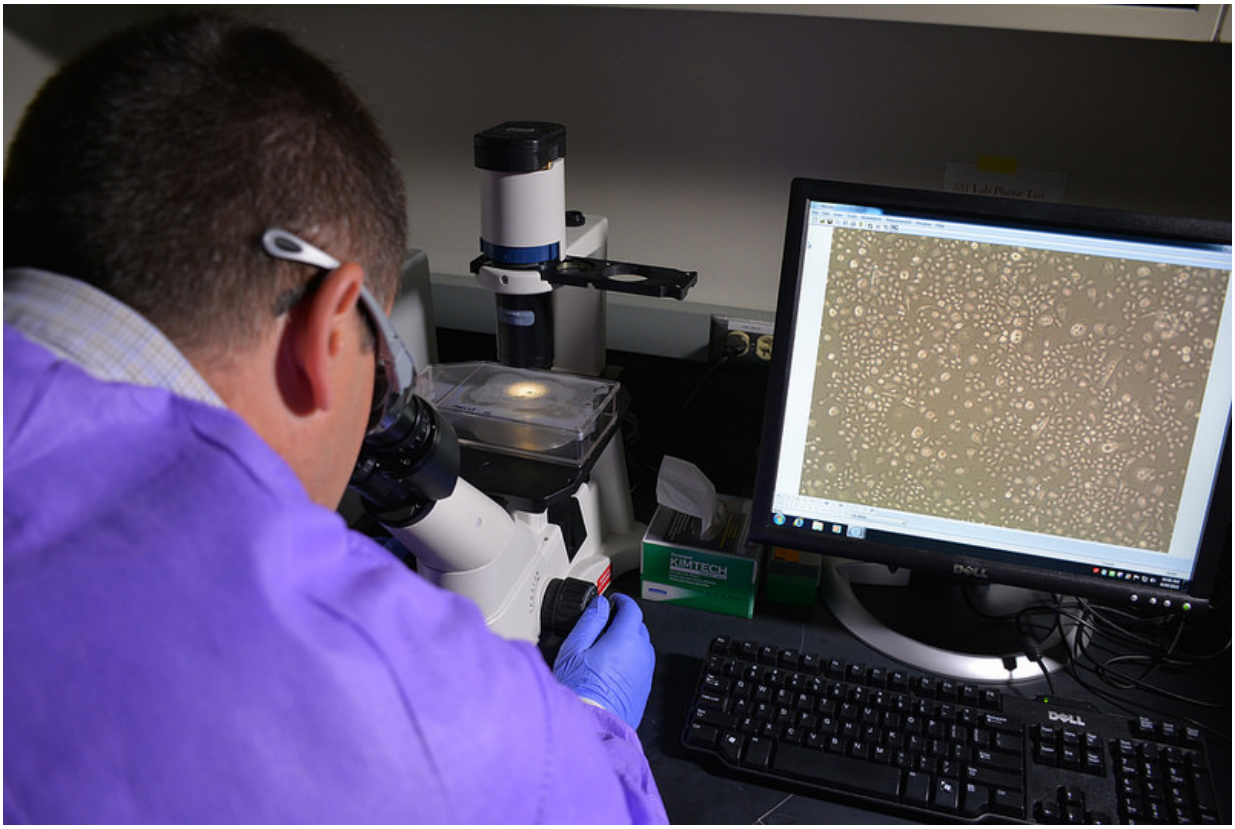


PNNL Microbiologist Josh Powell combines specially cultured rabbit lung cells with anthrax spores to get a better understanding how rabbit anthrax data compares to findings from human cell cultures.

Additional biochemical tests revealed that nutrients in the standard culture media provide an extra, unnatural fuel that makes spores germinate faster than would likely happen in the natural lung.

"These finding have implications for how we study pathogens within in vitro cell systems," said Powell. "Understanding the impacts of the methodology ensures we get the best data we can from both species on specific rates of spore intake or dose, clearance, germination and proliferation in a lab setting."

Researchers hope to reproduce this study using the more virulent strain at DHS's National Biodefense Analysis and Countermeasures Center in Frederick, Md., rather than the similar but milder Sterne strain used in this study, which is virtually unable to cause illness in people or animals.



Human lung cells magnified 200 times. PNNL microbiologist Josh Powel studies how normal human lung cells react differently to anthrax spores than the cancerous cell lines typically used in research.

Predicting to protect

In the next phase of the project, researchers will put this experimental data into a computational model to more accurately predict outcomes of

anthrax exposure. For instance, a model based on primary cell data may calculate how much time doctors have to initiate treatment, how many spores are likely needed to cause disease or mortality in humans, or be able to determine if there is a "safe" level for exposure or a required level of cleanup of a contaminated area.

Once the models are refined with data from the latest experiments, those numbers will be checked against animal data to see if they are indeed predicting outcomes accurately. The models could also potentially speed future drug design.

Researchers hope these fundamental findings and models can be applied to other diseases related to inhaled pathogens, such as the flu or SARS coronavirus. "This is an investment that may eventually help officials triage, treat and influence drug discovery for these lung illnesses," said Powell.

More information: "Bacillus anthracis spores germinate extracellularly at air-liquid-interface in an in vitro lung model under serum-free conditions." DOI: 10.1111/jam.12872

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