Bacteria Evolving:

Tracing the Origins of a MRSA Epidemic

STUDENT VERSION

PASSAGE THREE

Testing the Hypothesis

Scientists' initial hypothesis was that the speG gene was the cause of spermidine-resistence in USA300. Spermidine is a natural antibiotic given off by your skin when you have a cut or abrasion. They reasoned that the speG gene in USA300 makes it completely resistant to the killing power of spermidine.

Now they had to test this hypothesis. They wondered: What if they could disable the speG gene in USA300? What would happen then? Would USA300 still be resistant to spermidine?

IN THE LABORATORY

At the start of their experiment, scientists pipette knock-out samples of the speG gene onto live human skin cells growing in vials at Columbia University Medical Center in New York City.

To conduct this test, the scientists studied the effect of spermidine both on a "wild type" USA300 and on a "knockout" USA300, in which the original speG gene is either replaced by a non-functioning mutant copy of the gene, or the gene is deleted from the genome altogether. They expected that the modified version of USA300 would not be able to alter or neutralize spermidine.

First they had to prepare the knockout USA300. How could they get the bacteria to take in the foreign DNA with the modified gene? Well, as the scientists know, bacteria are very good at adopting foreign DNA into their genome. They do it all the time. They researchers decided to use the bacteria's own process of transformation. They placed USA300 bacteria in a medium that contained the mutant speG gene. Then they used small electric shocks to stimulate the USA300 bacteria to open pores in their outside cell membranes and take in the foreign DNA. This way, they successfully got some of the bacteria to replace their original speG gene with a non-functioning version.

This allowed them to run side-by-side tests. They recreated the conditions of a skin infection by growing human skin cells in a culture. To each sample they added some spermidine and then introduced the two versions of USA300, the wild type and the knockout. The results supported their hypothesis. The knockout

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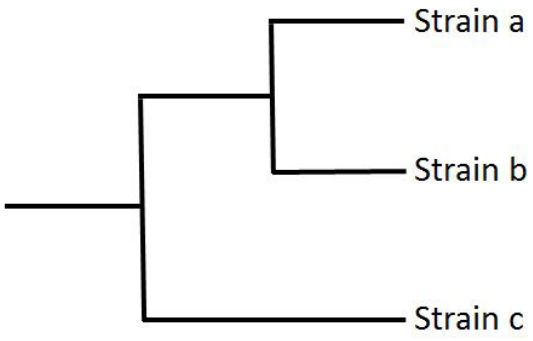
Testing the Hypothesis

bacteria fared much worse than the wild group with functioning speG genes. Without a defense against the spermidine, the proportion of mutant USA300 bacteria that survived was much smaller. The difference was clear: it was the speG gene that gives USA300 protection from killing by human skin. They also found that the speG gene allows USA300 to better attach itself to human skin.

Next, scientists wondered when USA300 first acquired the speG gene. To find out they created a phylogenetic tree of MRSA strains. A phylogenetic tree, or cladogram, is a branching diagram that shows the relationships among organisms based on comparisons of physical or molecular characteristics. The diagram indicates which groups are most closely related. The order of branching points on the tree can be used to infer the order of historical events in a particular lineage. Scientists used this technique that the acquisition of the speG in the USA300 lineage probably occurred around 1997, which coincides with the first outbreaks of community-acquired MRSA.

But there was one more piece to the puzzle. How did USA300 get the speG gene? Where did it come from? They knew the answer probably lay somewhere in the vast microbiome that exists on human skin.



CLOSE RELATIVES
This simple phylogenetic tree shows that strain a and strain b are more closely related to each other than either is to strain c.

STOP AND THINK

Based on the text:

- **What experiments did the scientists design to find out whether the speG gene is responsible for USA300's unique characteristics? Create diagram that shows the design of the experiment. How does this compare to your answer from Passage 2?**
- **What question did the scientists answer by building a phylogenetic tree?**

Looking ahead:

- **If USA300 acquired the speG gene through DNA transfer, what steps might the scientists take in order to find the source of that gene?scientists design to find out whether the speG gene is responsible for USA300's unique characteristics?**