Illuminating Science Addresses

TB THREAT To Global Health

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Writer

Robert Abramovitch, Michigan State University assistant professor of microbiology, is synthesizing new drugs to treat tuberculosis, an illness raising concerns because of its growing resistance to antibiotics.

A quick Internet search of "tuberculosis" (TB) returns thousands of hits that describe the devastating disease. News articles, blog posts and images capture the struggle of people from all over the world, and the short video segments are notably alarming. They tell the story of millions contending with the reality of an antibiotic-resistant TB infection: compromised health, derailed dreams and serious worries about the future.

Robert Abramovitch, Michigan State University (MSU) AgBioResearch scientist, is leading an innovative approach to develop a drug treatment that could affect the fate of billions and help prevent the spread of antibiotic-resistant TB, a growing burden to both the United States and countries abroad. His commitment to connecting fundamental research with applied results is underscored by the need for creative, science-based solutions that address this daunting health concern.

TB: An inventive, international foe

The Centers for Disease Control and Prevention (CDC) describes TB as "one of the world's deadliest diseases" and, in 2013, labeled *Mycobacterium tuberculosis* (MTB) — the bacterium that causes the disease — a serious threat in the struggle to control antibiotic resistance.

Symptoms of TB disease include feelings of sickness and weakness, weight loss, fever and night sweats. When TB-causing bacteria target the lungs, the infected person experiences chest pain and coughing so severe that it may lead to the expulsion of blood and sputum.

These side effects are characteristic of the active form of the disease; its second form, however — latent TB — has no symptoms and can live silently in the human body for decades. Latent TB becomes active once the immune system is compromised.

The CDC estimates that more than 2.3 billion people are infected with latent TB – that's one-third of the global population.

"When MTB infects humans, our immune system walls off the infection by building a granuloma — a tumor — around the bacteria, which is why you seem totally healthy if you have latent TB," said Abramovitch, MSU assistant professor of microbiology and molecular genetics. "But the granuloma doesn't kill the bacteria — instead, the bacteria change their physiology so they can survive inside."

Abramovitch explained that MTB's ability to do this is no small feat, but rather an extremely difficult biochemical trick. The bacteria sense environmental cues and then substantially slow their growth, changing the way they use and make energy to survive inside the granuloma. Abramovitch hypothesizes that oxygen plays a pivotal role in this process.

"MTB needs oxygen to grow," he explained.

"We believe it has the ability to sense that the oxygen level around it is decreasing — a state known as 'hypoxia.' When it senses that the environment has become hypoxic, that's its cue to say, 'OK, I need to hunker down.'"

The bacteria remain dormant — having optimized their ability to survive for years in a stressful environment — until they sense that the environment favors growth. Once that happens, MTB initiates a genetic pathway that leads to the active state of the disease.

Abramovitch is working to develop pharmaceutical drugs that will prevent the bacteria from sensing changes in the environment and establishing a dormant state.

"Normal TB takes about six months to treat and requires daily antibiotics," he explained. "If you've ever taken antibiotics for two weeks, you know you're likely to miss at least one dose – everyone does. The problem is that when people don't stringently complete the six-month drug course, they can breed drug-resistant TB. The dormant, slow-growing bacteria are harder to kill, resulting in the long treatment course required to cure TB."

Inhibiting the ability of the bacteria to establish dormancy may shorten the course of antibiotic treatment, thus eliminating the disease more quickly and reducing the emergence of drug-resistant TB, Abramovitch said.

Breaking ground in an area of global health that hasn't seen drug advancements in more than 40 years is challenging, but Abramovitch is relying on the help of a protein that has a little Spartan spirit to do just that.

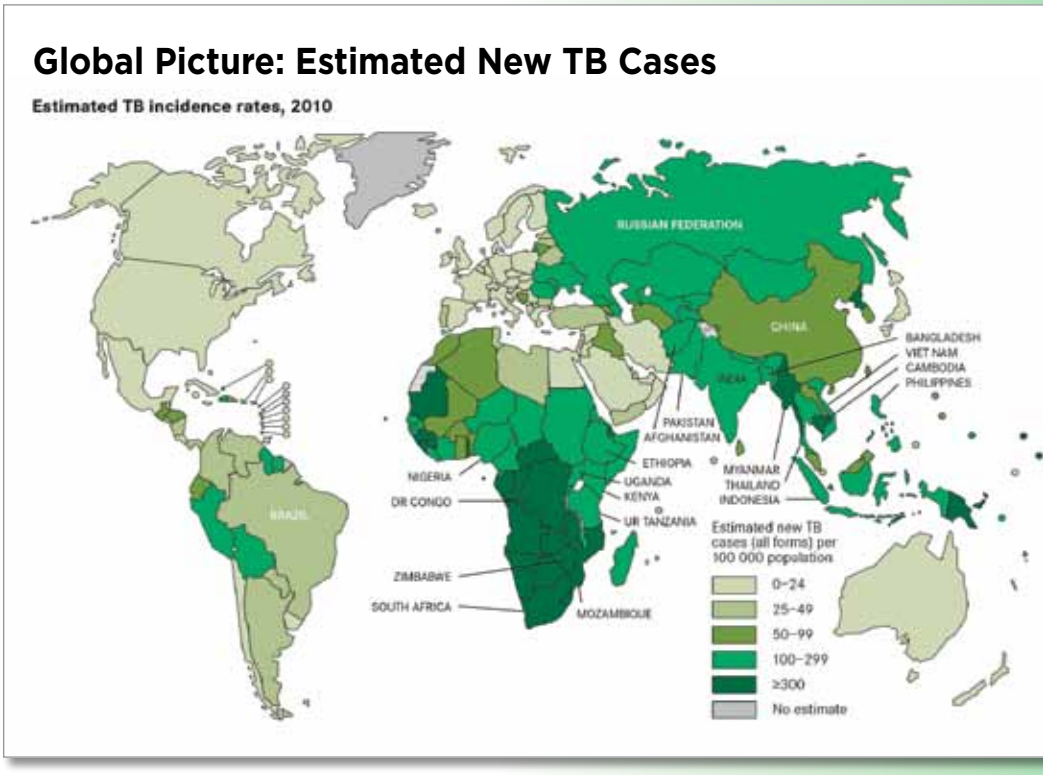
Biosensors point to glowing solutions

In 2012, Abramovitch was named a Bill and Melinda Gates Foundation Grand Challenges Explorations awardee. This initiative recognizes scientists who take a non-traditional approach to solving persistent, global health challenges; the winners receive an initial grant of $100,000 (successful projects can receive up to an additional $1 million). With the grant funding, Abramovitch used a green fluorescent protein (GFP) biosensor to search for new TB treatments.

To identify compounds that disrupt the hypoxia-sensing ability of MTB, Abramovitch employs an experimental method called high-throughput screening to quickly screen thousands of compounds in large batches. To do this, however, Abramovitch had to genetically engineer a biosensor he could insert into MTB. The biosensor is a green-glowing protein that, in a test tube, signals that MTB is transitioning to a dormant state.

"MTB has to adapt to low oxygen, but if you just look at its cells in a test tube, you can't tell if they're adapted for regular oxygen levels or low oxygen levels because the bug doesn't have a natural signal that indicates its physiological status," Abramovitch explained.

The biosensor acts as a synthetic signal, enabling Abramovitch to run high-throughput screens in the lab. The biochemical marker doesn't have any fluorescence when oxygen levels are normal, but it begins to glow green as the levels drop and MTB initiates hypoxia



ABOVE: The World Health Organization estimated that there were 8.8 million new cases of tuberculosis disease in 2010. The map illustrates the global occurrence and concentration of those cases.

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>
> *— Robert Abramovitch*

(Continued on page 32.)

(TB threat to global health — continued from page 17.)

LEFT: Doug Buhler, MSU AgBioResearch director and senior associate dean of research for the College of Agriculture and Natural Resources, explained the importance of high-risk, long-term research like Abramovitch's, noting that many of today's advancements can be traced to the research of decades past.

adaptation pathways.

Next, he took the MTB biosensor strain and conducted a drug screen to find compounds that would turn off the green signal, disabling MTB from sensing the hypoxia cue to transition to dormancy.

Collaborating with Harvard Medical School in Boston, Abramovitch and his team used liquid-handling robots to prepare 800 drug-screen plates, each holding 384 compounds. Then, in a newly built, high-throughput screening facility at MSU, his team tested about 280,000 compounds for the ability to inhibit MTB hypoxia sensing and survival.

The lab is specially outfitted so scientists can work safely with infectious MTB and other airborne agents.

Doug Buhler, director of MSU AgBioResearch and senior associate dean of research for the College of Agriculture and Natural Resources, said he has strong confidence in Abramovitch's work and emphasizes the value of innovative, untraditional research that leads to important discoveries.

"It's actually very rare for people to do high-throughput screening with infectious MTB," Abramovitch noted. "Usually people use less virulent, attenuated strains, but these strains are missing important virulence mechanisms that make MTB a successful pathogen. I believe there are benefits to using real, infectious MTB, even though it's an unconventional thing to do.

"The screen had two discovery arms," he said. "We found compounds that targeted the biosensor specifically, turning off the green marker, but we also had a bonus benefit — which may be better than the first — which is that we found compounds that just killed MTB."

Abramovitch explained that he and his team will now conduct additional confirmation experiments and then test compounds of interest to understand how they turn off the hypoxia-induced pathway or kill MTB altogether. These findings will help him develop drugs to use in animal models during follow-up validation studies.

Doug Buhler, director of MSU AgBioResearch and senior associate dean of research for the College of Agriculture and Natural Resources, said he has strong confidence in Abramovitch's work and emphasizes the value of innovative, untraditional research that leads to important discoveries.

"His research is very important because of the complicated and serious nature of the tuberculosis problem," Buhler explained. "I once heard Ian Gray [former MSU AgBioResearch director and MSU vice president for research and graduate studies] make a statement that has stuck with me: 'We support fundamental research with an intended outcome.' That's a really big part of who we are and what we do. It's also a statement that describes Abramovitch's work very well: this is high-risk, long-term research that will have a big impact on global health in the future."

Robert Hausinger, interim chair of the MSU Department of Microbiology and Molecular Genetics, said Abramovitch is beginning to make significant headway on a topic of growing concern.

"Abramovitch tells a wonderful story and combines some really nice genetics, metabolism and cell physiology to do work that is fantastic and exciting," Hausinger said. "It's important to note that his facility allows him to do work that other researchers in the nation — in the world — can't do. There are only a few of these facilities in which people are working with MTB, and he's very rapidly come to the forefront of the field because of the experiments he's able to do there."

Addressing problems with applied discoveries

Abramovitch vividly recalls the crossroad he faced after earning his doctorate: "By the time I had finished my Ph.D., I had made a lot of big, basic research discoveries," he said. "I looked at them and thought, 'Well, these are good, but I don't know how this is going to be applied in the real world.'"

Desiring to apply his findings, he began to look at some of the world's greatest problems. TB stood out as a prominent issue that would require new, science-based solutions to address it adequately.

"From the very start, I took the biosensor approach because it created an opportunity for me to do basic research that could be readily translated into a real-world, drug discovery platform," he concluded. "The Gates Foundation and MSU AgBioResearch funded these projects because they're non-traditional and connect basic research with applied research. You have to try new things — you never know what's going to work. And even though TB drug discovery hasn't seen many new advances, we still have to try."

Abramovitch plans to publish more details about the findings of his drug screen; for more information, visit mmg.msu.edu/abramovitch.

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