Pouncing on Food Pathogens: It Takes a Planet!

PEGGY GREB (D2161-1)

At the Plant Mycotoxin Research Unit in Albany, California, molecular biologist Jong Kim (left) and research leader Bruce Campbell inspect assays of natural compounds that can significantly improve the fungicidal activity of commercial antifungal agents.

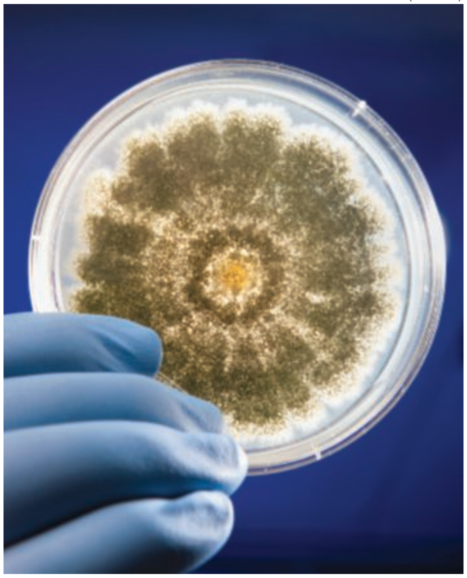
Fungi that produce chemicals harmful to people, animals, or plants respect no boundaries. *Aspergillus flavus,* the fungus that makes cancer-causing compounds known as "aflatoxins," is one such organism. This mold is a threat to the wholesomeness of popular tree nuts worldwide, mainly almonds, pistachios, and, to a lesser extent, walnuts.

Agricultural Research Service scientists in Albany, California, have teamed up with colleagues halfway around the globe—in Moscow—to explore new strategies for destroying *A. flavus.* Their anti-*Aspergillus* tactics might help quell other troublesome fungi, as well. That is why this collaboration with the Russian Research Institute of Phytopathology in Moscow encompasses not only *A. flavus* but also several other key fungal foes. Targeted microbes include, for instance, *Fusarium culmorum* and *Bipolaris sorokiana,* both of which can cause root rots and other problems, and *Alternaria alternata,* which causes leaf spot disease of some crops.

Research leader Bruce C. Campbell, who heads the ARS Plant Mycotoxin Research Unit at Albany, developed the international collaboration to quicken discovery of natural compounds that could work in concert with known fungicides. Such pairings would deliver a one-two punch, with the natural compound making the target fungi more vulnerable to the fungicide.

Studies at Albany, started in 2004 by Campbell and by ARS molecular biologist Jong H. Kim, provide strong evidence to support this intriguing concept. By reducing the amount of fungicides commonly used today, the strategy may prove to be less costly and more environmentally friendly than conventional approaches, Campbell says.

PEGGY GREB (D2162-1)



Petri dish containing the fungus *Aspergillus flavus*. This common fungus is a concern because it produces carcinogenic aflatoxins, which can contaminate certain foods and cause aspergillosis, an invasive fungal disease.

Campbell, Kim, and their collaborators have published their findings in *Applied Microbiology and Biotechnology, Biochemical and Biophysical Research Communications, FEMS Microbiology Letters, Fungal Biology, Letters in Applied Microbiology, Mycopathologia, and World Mycotoxin Journal.*

At the Moscow institute, scientists are rigorously testing the concept in studies coordinated by Vitaly Dzhavakhiya and Larisa Shcherbakova. In their experiments, the team has determined, for example, that a very small amount of thymol, a natural compound from thyme, when added to Folicur (tebuconazole), a commercial fungicide, "was about twice as effective in reducing growth of *A. alternata* than when the fungicide and thymol were applied singly," Shcherbakova reports.

In related work, the team is testing enzymes produced by beneficial, edible mushrooms, determining the enzymes' prowess as "biodestroyers" of aflatoxins. Several mushrooms in the *Phoma* genus appear to be promising sources of aflatoxin-degrading enzymes.

Both the U.S. and Russian teams are also curious to know how, precisely, the most promising natural compounds and enzymes succeed in disrupting the inner workings of harmful fungi. In particular they want to discover how the compounds and enzymes reduce a fungus's ability to grow, to defend itself against fungicides, and—in the case of certain *Aspergillus* species—to produce aflatoxins.

The project is one of many collaborations with the former Soviet Union that are administered by ARS's Beltsville, Maryland-based Office of International Research Programs. In fiscal year 2010, the U.S. Department of State provided about $1 million to fund these collaborations.

Other ARS international partnerships target other problematic microbes. Here's a quick look at two of those projects.

High-Tech Tactics To Detect Pathogen Sources on Fresh Produce

Before that crisp head of lettuce or juicy apple reaches your hands, it passes through a series of inspections to make sure it's good enough for you to eat. From color to shape to size, the produce is evaluated against a wide variety of criteria before it arrives in your local grocery store.

Number one on that list? Food safety, because the qualities you love about fresh produce won't matter one bit if you get sick. That's why biophysicist Moon Kim is working hard to develop new technologies that can help food safety inspectors detect harmful pathogens on the fruits and vegetables everyone enjoys.

Kim works with ARS agricultural engineer Kevin Chao, biomedical engineer Alan Lefcourt, and others at the Environmental Microbial and Food Safety Laboratory in Beltsville, Maryland. They have been recognized for their advances in food safety technology. The researchers first developed a high-speed, multispectral line-scan imaging system for use on poultry carcasses, which has been applied to identifying unwholesome birds and detecting traces of feces that could transmit harmful pathogens to humans. They are modifying the technology for use on fresh fruits and vegetables, which can also contain traces of feces from manure used to fertilize the soil. (Read more about this in "Machine's Eye View of Poultry and Produce," *Agricultural Research,* January 2007.)

Now, through a formal agreement with South Korea's Rural Development Administration, Kim and colleagues are collaborating on applications of this groundbreaking technology for use in South Korea. "Food safety and security is a global issue," explains Kim. "Ensuring that food supplies are free from pathogens and disease benefits everyone, worldwide."

For the past 4 years, ARS and Korean scientists have been collaborating to improve the sensing technology for fresh produce. They recently developed and patented a multitask imaging system capable of examining quality and safety attributes of apples. The new technology scans 3-4 apples per second, providing efficient and effective inspection of defects and fecal contamination. Details of this research have been published in *Sensing and Instrumentation for Food Quality and Safety*.

Kim and colleagues are currently looking at ways to improve the new technology, such as developing methods to examine the entire surface of a round object. With the researchers' continued dedication, consumers can rest assured that the food they eat will be safe and secure.

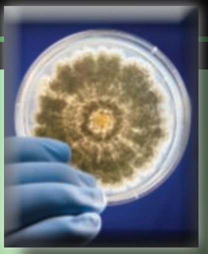
Foreign Beef: "Microbial Profiling" System Gets an OK from Scientists

When a side of beef is neatly carved into steaks and roasts, bits and pieces of meat trimmed from these familiar retail cuts are left over. In the meatpacking industry, they're known, not surprisingly, as "trim."

In the United States, there's a high demand for trim that can be used to make lean ground beef, perfect for burgers, meatloaf, meatballs, and other favorites. In fact, the U.S. demand for lean ground beef exceeds our domestic supply. That's why, in part, we import about 3 *billion* pounds of beef and veal every year.

Several years ago, questions were raised as to whether America's procedures for monitoring the safety of imported beef were adequate for detecting pathogens in trim. "Foodborne pathogens and their reported incidences aren't necessarily the same from one part of the world to the next," notes ARS microbiologist Joseph M. (Mick) Bosilevac.

An example: *Escherichia coli* O157:H7 is the leading species, or serotype, in *E. coli*-associated foodborne illness in the northern hemisphere. But in the southern hemisphere, other toxin-producing *E. coli* serotypes such as O111 have also been associated with outbreaks of foodborne illness.



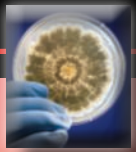
What's more, when imported beef and domestic beef are combined to make a lean ground beef product, "traceback" becomes much more complex. Traceback, in which sources of food contamination are, if possible, traced back to their point of origin, is a standard part of investigations that occur during and after major outbreaks of foodborne illness.

"The intent of our study was to find out whether U.S. microbiological profiling of imported beef trim adequately addresses the potential differences between foreign and domestic beef in terms of cleanliness and safety, or what we describe as 'hygienic status and pathogen presence,'" says Bosilevac.

For the study, Bosilevac and coresearchers examined 1,186 samples of beef trim from the United States and from Australia,

To determine the presence of *Salmonella, Listeria,* and non-O157:H7 *E. coli* in beef samples, technician Greg Smith (right) collects surface samples from boneless beef trim as microbiologist Mick Bosilevac prepares a sample for eventual analysis.

STEPHEN AUSMUS (D2159-11)



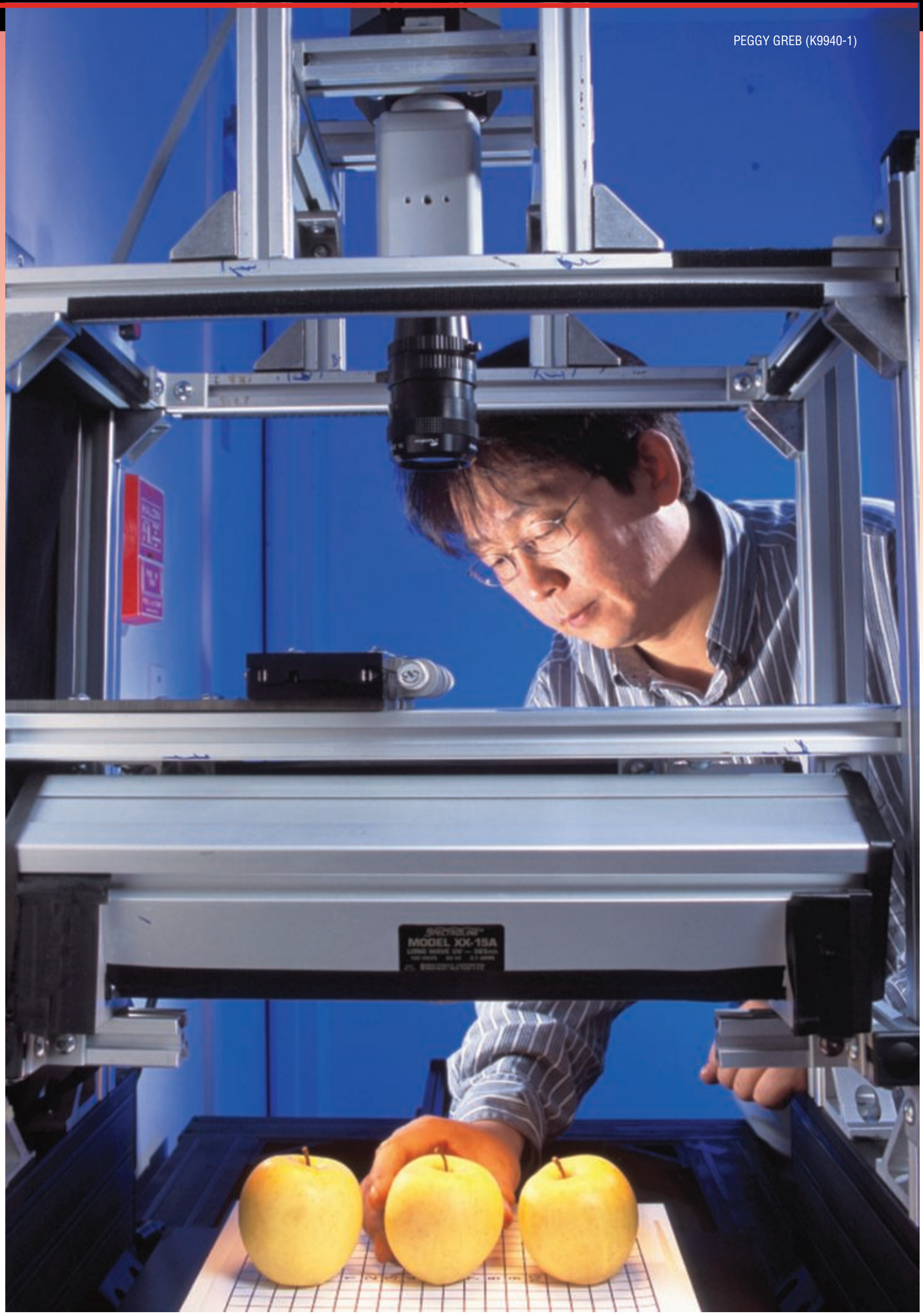
New Zealand, and Uruguay—three nations that provide more than half of America's beef imports. The researchers looked for contaminants such as aerobic bacteria, *Staphylococcus aureus, Campylobacter, Salmonella, Listeria,* and *E. coli*—specifically the close relatives of *E. coli* O157:H7 that can cause severe foodborne illness.

"Our results indicate that the pathogen-monitoring procedures used in the United States today are adequate for evaluating the safety of imported beef trim," says Bosilevac. He's based at the ARS Roman L. Hruska U.S. Meat Animal Research Center (USMARC) at Clay Center, Nebraska.

Bosilevac and coinvestigators Michael N. Guerini, Dayna M. Brichta-Harhay, and Terrance M. Arthur at Clay Center; and Mohammad Koohmaraie, formerly with USMARC, documented the research in an article that appeared in a 2007 issue of the *Journal of Food Protection.*

The study led to an informal, ongoing collaboration in which Bosilevac and research leader Tommy L. Wheeler have presented information about USMARC's leading-edge technologies for detecting and identifying foodborne pathogens to colleagues at several of Uruguay's national laboratories and at the Instituto Nacional de Investigación Agropecuaria, the Uruguayan counterpart of the U.S. Department of Agriculture. Food safety specialists from Uruguay have also come to USMARC to see this science in action.

The beef-trim research was funded in part by the Beef Checkoff, a producer-financed program of beef-related promotion and research.—By **Marcia Wood,** ARS, and **Stephanie Yao,** formerly with ARS.



This hyperspectral imaging system, being used by biophysicist Moon Kim, takes pictures at different wavelengths simultaneously. Three-dimensional images are created from the process, and researchers can then choose the wavelengths best suited for spotting fecal contamination or cuts and bruises in agricultural products.

This research supports the USDA priority of ensuring food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

Bruce C. Campbell and Jong H. Kim are in the Plant Mycotoxin Research Unit, USDA-ARS Western Regional Research Center, 800 Buchanan St., Albany, CA 94710; (510) 559 5846 [Campbell], (510) 559-5841 [Kim], bruce.campbell@ars.usda.gov, jongheon.kim@ars.usda.gov.

Moon Kim, Kevin Chao, and Alan Lefcourt are with the USDA-ARS Environmental Microbial and Food Safety Laboratory, 10300 Baltimore Ave., Beltsville, MD 20705; (301) 504-8450 ext. 245 [Kim]; (301) 504-8450 ext. 260 [Chao]; (301) 504-8450 ext. 258 [Lefcourt], moon.kim@ars.usda.gov, kevin.chao@ars.usda.gov, alan.lefcourt@ars.usda.gov.

Joseph M. (Mick) Bosilevac and Tommy L. Wheeler are in the Meat Safety and Quality Research Unit, USDA-ARS Roman L. Hruska U.S. Meat Animal Research Center, Spur 18D, Clay Center, NE 68933; (402) 762-4225 [Bosilevac], (402) 762-4221 [Wheeler], mick.bosilevac@ars.usda.gov, tommy.wheeler@ars.usda.gov. ✻