by Linda L. Leake

New Worlds of **Microbiological Testing**

Some people might say that microbiological testing of foods is in a slump. That's because there haven't been too many breakthroughs in this discipline for some time, according to Douglas Archer (dlarcher@ufl.edu), Associate Dean for Research, Florida Agricultural Experiment Station, University of Florida, Gainesville. "We are overdue for a jump in technology," he says. "We need to find a way to get away from selective enrichment. We need a real-time method to identify microorganisms that can be used in the field."

Nanotechnology, the study of materials measuring 100 nm or less, *holds the promise for helping researchers to develop a real-time testing method.*

Currently, scientists rely on two major technologies for microbiological testing: molecular and immunological, says Ann Marie McNamara (info@silliker.com), Vice President of Food Safety and Scientific Affairs, Silliker, Inc., Homewood, Ill. "Polymerase chain reaction (PCR) and immunoassays are the workhorses," she relates. "They are fast and highly accurate. PCR is more sensitive but more complex. Lateral flow tests are easy and fast. Industry always has a need for faster tests because of the perishable nature of food; however, current tests require growth of the organism to reach their detection limit, and you can only push bacterial growth rates so far. Speed can be a tradeoff for accuracy."

McNamara doesn't expect any new breakthroughs in testing methods within the next year. "Some methods are still in the developmental stages," she points out.

The* Tempo *pathogen indicator *system technology is based on a classic, well-established microbiological method called Most Probable Number.* Photo courtesy of bioMérieux

Breaking Out of the Slump

Nanotechnology, the study of materials measuring 100 nm or less, holds the promise for helping researchers to develop a real-time testing method, Archer believes. "Some scientists know how to link receptor molecules specific for the target they want to find, such as a bacterium or virus, with nanoparticles and a 'reporter' system—something that makes the target detectable," he says. "The result is a smart probe that can greatly amplify a single target hit on the nanoparticle."

Qingrong Huang (qhuang@aesop.rutgers.edu), Assistant Professor, Dept. of Food Science, Rutgers University, New Brunswick, N.J., uses nanotechnology to develop rapid and sensitive detection techniques for organisms like *Escherichia coli* O157:H7.

"The low infectious dose of *E. coli* O157:H7—less than 100 cells—makes the development of rapid and sensitive detection methods necessary to prevent occurrence of widespread outbreaks," Huang says. "Traditional detection methods for trace amounts of bacteria require amplification of the target organism. Recent progress in nanotechnology leads the way for

development of novel detection methods with higher sensitivity to eliminate the laborious and time-consuming amplification process."

Efforts to improve the stability, sensitivity, and reproducibility of various bioassays for pathogenic cell detection have included developing new probe materials of high stability and quantum yield. Semiconductor quantum dots (QDs) are one example of promising materials that may be used to improve the bacterial detection limit. "These QDs make the detection of a single pathogenic cell possible," Huang says.

Boasting unique optical profiles, QDs have size-dependent tunable photoluminescence with broad excitation spectra and narrow emission bandwidths. These characteristics allow the simultaneous excitation of particles of different sizes at a single wavelength. In addition, their high photobleaching threshold renders continuous or long-term monitoring of slow biological processes possible.

Huang's group has successfully developed a simple bioconjugation procedure that allows the attachment of a water-soluble cadmium tellurium QD to an antibody. "We have QD-conjugated anti-pathogen antibodies of different colors in our lab, including anti-*E. coli* antibody," Huang relates. "Now QDs become promising probe materials in the development of antibody-based immunosensors with high stability, sensitivity, and reproducibility."

Star Trek Technology

Scientists at Purdue University, West Lafayette, Ind., have created a handheld sensing system that they say is similar to the "tricorder" that the characters in *Star Trek* used to analyze the chemical components of alien worlds. The prototype system, called *Mini 10*, holds promise for applications on Planet Earth, including testing foods for bacterial contaminants, according to R. Graham Cooks (cooks@purdue.edu), the research leader and Henry Bohn Hass Distinguished Professor of Chemistry at Purdue.

The instrument is a miniature mass spectrometer combined with a technique called desorption electrospray ionization (DESI), also developed by Cooks' team. "Conventional mass spectrometers

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***The* 3M Petrifilm Environmental *Listeria* Plate** *not only identifies the presence or absence of* Listeria, *but provides a quantitative indication of the level of* Listeria *contamination.*
Photo courtesy of 3M Microbiology

analyze samples that are specially prepared and placed in a vacuum chamber," Cooks says. "The key DESI innovation is performing the ionization step in the air or directly on surfaces outside of the mass spectrometer's vacuum chamber."

Unlike conventional mass spectrometers, which typically weigh more than 300 lb, the battery operated, handheld device weighs about 20 lb and can be used in the field.

"We like to compare it to the 'tricorder' because it is truly a handheld instrument that yields information about the precise chemical composition of samples in a matter of minutes without harming the samples," Cooks explains.

Using the *Mini 10*, the researchers look for compounds that indicate the possible presence of a particular substance. If these indicators are found, the equipment performs a more in-depth analysis to determine the exact chemical structure.

While identifying bacteria is the current microbiological research and development focus, Cooks says the instrument will be applicable for identifying all types of microbes. One development is to determine if biomarkers in the spectra can always be seen independently of the nature of the matrix.

Cooks explains that the nature of the matrix (growth medium or substrate) also affects the mass spectrum. "So one needs to recognize and perhaps create a library of characteristic ions the microorganism features, even though there are features present due to the medium. So far, in controlled microorganisms grown in the lab, this is not a problem, but it could be in a more-diverse set of samples."

The portable mass spectrometer should be available commercially in about 18 months, Cook predicts. "It is not a great quantitative device, but it provides information in about five seconds. The main advantage will be fast in-situ analysis in the field and at the plant. If the instrument determines there is cause for concern, the operator can then proceed further with more detailed testing."

Two Indiana-based startup companies have been established: Prosolia Inc., in Indianapolis, has commercialized the DESI technique, and Griffin Analytical Technologies LLC, in West Lafayette, has commercialized miniature ion-trap mass spectrometers.

Testing Produce

The food industry needs a

Microbiological Testing Products Released

Several companies now offer testing products that can screen for a variety of microbes.

• Earlier this year, bioMérieux, Marcy l'Etoile, France (+33-04-7887-2000, www.biomerieux.com), launched *Tempo*®, touted as the first quality indicator system in the food industry, in the United States. The system includes quality indicators for total viable counts, total coliforms counts, generic *Escherichia coli* and *Enterobacteriaceae,* which are useful for determining overall product hygiene and indicating product spoilage. Through automation, *Tempo* takes the established, labor-intensive Most Probable Number (MPN) pathogen monitoring method and standardizes numerous preparation steps, interpretations, and test results. The outcome is said to be a fast, accurate method that is more reliable than MPN.

• Hanson Technologies, Inc., Carlisle, Pa. (717-245-9890, www.hansontechnologies.com), offers *Hanson Safe Vegetable Screening System*, which uses the company's patented and patent-pending *UltraRapid*™ technology for pathogen detection in 100% of fresh-cut produce in two hours or less without the need to culture the bacteria. The system plugs directly into the food production supply chain by analyzing the wash water after an entire batch of product is washed. Capabilities include the simultaneous screening for multiple pathogens, allergens, and toxins, including *E. coli* and *Salmonella*.

• Health Canada, the federal agency that oversees food safety in Canada, has recently validated protocols for using the *Bax*® system from DuPont Qualicon, Wilmington, Del. (800-863-6842, www.qualicon.com), to detect *E. coli* O157:H7 in fruits, vegetables, dairy, meat, animal feed, environmental samples, and dry products. In 2004, the system received AOAC certification for testing of *E. coli* O157:H7 in raw beef, apple cider, and orange juice. USDA FSIS adopted *Bax* to screen for *E. coli* O157:H7 in raw ground beef and beef trim.

• Last month, 3M Microbiology, St. Paul, Minn. (888-364-3577, www.3m.com/microbiology), announced that AOAC has validated its *3M Petrifilm Environmental* Listeria *Plate*, first released in 2004, for the detection of *Listeria* on environmental surfaces in food processing plants. The product provides a quantitative indication of the level of *Listeria* contamination, instead of simply identifying the presence or absence of the organism. The results are available in 27-31 hr after sample collection, with no enrichment step required.

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microbiological testing program to test for *E. coli* O157:H7 in leafy greens, similar to the one used to test for the microbe in ground beef, says Michael Doyle (mdoyle@uga.edu), Director and Regents Professor of Food Microbiology, University of Georgia, Griffin.

Doyle is referring to an initiative by the ground beef industry in 2003 to screen for the *E. coli* O157:H7 pathogen in raw beef ingredients before they are ground into finished product. This testing program, combined with other food safety interventions implemented by ground beef processors, has led to a remarkable reduction in *E. coli* O157:H7 contamination of ground beef.

"In each of the last three years, USDA's Food Safety and Inspection Service has found that just 0.17% of the overall raw ground beef samples from all sources were positive for *E. coli* O157:H7," Doyle says. "Previously, up to 0.8% of the samples were positive. Beef trim testing has been a successful intervention, and the same concept would be beneficial for the fresh-cut salad industry by prescreening for *E. coli* O157:H7 in leafy green ingredients prior to processing."

For the effective testing of produce, a practical, rapid test for *E. coli* O157:H7 is needed, one that takes 12 hr or less to conduct and does not interrupt the production process, Doyle emphasizes. "An important consideration is that the test must be validated for sensitivity, specificity, and accuracy in detecting the pathogen. This is a tremendous opportunity for an intervention that would detect an exceptional occurrence of *E. coli* O157:H7 contamination and enable the processor to prevent contaminated leafy greens from being processed into bagged ready-to-eat products."

Detecting Spoilage Organisms

The needs for microbiological testing are expanding beyond pathogens, says Phil Elliott (pelliott@fpa-food.org), Director of Microbiology, Grocery Manufacturers/Food Products Association, Washington, D.C. Spoilage is one new area of focus.

"*Alicyclobacillus* species and *Propionibacterium cyclohexanicum* are heat-resistant acidophilic bacteria that can grow and produce off flavors and odors during the spoilage of juices, teas, sport drinks, etc.," he elaborates. "I believe the next new area in microbiological testing will be early and rapid detection of food spoilage.

"This may include more accurate ways to predict spoilage and more rapid ways to detect and identify spoilage organisms. Many of the companies that currently make rapid detection methods for pathogens in food may find another niche in rapid methods for detection of food spoilage microorganisms." **FT**

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