Pathogen Detection at the *Speed of Light*

RAPID METHODS HAVE REVOLUTIONIZED RAW MATERIALS TESTING, BUT CHALLENGES REMAIN

Many people believe nothing is certain in life but death and taxes. Daniel Y.C. Fung, PhD, professor of food microbiology at Kansas State University, adds another certainty to the list: "Food processors must get accurate results from tests to detect pathogens in raw materials. This holds true regardless of the technology employed, the time involved, or the cost. A rapid test giving bad results is not good whatsoever."

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COVER STORY BY **LINDA L. LEAKE, MS**

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Accuracy may be the top priority of microbial testing, but in light of the fast-paced global food system, speed is critical to every processing plant's efficiency and profitability. While there are still no magic bullets delivering immediate results, there are many new and exciting rapid methods to test for bacteria, yeast, and molds in raw and processed food samples, Dr. Fung says. "Different microbiological tests measure different attributes of the cell mass, numbers, metabolites, and genetic materials, so it is really difficult to single out the significance of one test over another."

Scientists currently rely on two major technologies for microbiological testing, molecular and immunological; both are considered highly accurate and fast. Of these, polymerase chain reaction (PCR) and immunoassays are the key methods available, respectively.

"Real time PCR systems and multiplex PCR systems offered by several high-tech companies are making great advances in rapid identification of pathogens," Dr. Fung says. "A number of eight-hour tests are on the market for a number of pathogens, including *E. coli* O157H:7, which provide results during one work shift." Dr. Fung himself developed what's known as the Fung Double Tube (FDT) system, a six-hour test that has been used to detect *Clostridium perfringens* in ground beef.

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—Daniel Y.C. Fung, PhD, Kansas State University

MICRO HITS THE BIG TIME

The development of such tests is an indication of the market's vitality. According to *Food Micro—2008 to 2013*, a market report published by Strategic Consulting, Inc. (SCI; Woodstock, Vt.), over 738 million food industry microbiological tests, with a market value exceeding $2.06 billion, were completed in the global market in 2008.

"The food sector represents the largest market segment within the industrial microbiology market...almost 50% of the total market," says Thomas Weschler, MBA, SCI's president. "The food sector is more than double the size of any other industrial segments, including the pharmaceutical, beverage, environmental, industrial process, and personal care products sectors."

Since 1998, the market value for food microbiology has grown significantly, experiencing an annual growth rate of 8.7%. "Based on SCI research, the food microbiology testing market is expected to grow to 969.2 million tests in 2013, with a market value approaching $2.4 billion," Weschler says. "This represents a projected annual growth rate of 5.6% in testing volume."

Much work is being done to meet that demand. A variety of nanotechnology tests and procedures for image analysis of colonies are being developed, Dr. Fung adds. "These are not yet ready for commercial use but in due time may be available in food microbiology laboratories."

There are some limitations with current rapid micro tests, Dr. Fung says. Some instruments and systems, like Fourier transform infrared (FTIR) spectroscopy, require only a few minutes to precisely identify a colony on an agar plate but are very expensive. Some rapid micro tests do not measure cell viability, an important factor in food microbiology. And some slow-growing organisms, such as yeast and mold, are difficult to detect using rapid methods.

"At least 50 companies throughout the world are working on a myriad of systems, and we are seeing new breakthroughs relative to rapid micro tests every day," Dr. Fung says. "In the next three years, new ideas and procedures will emerge on many fronts."

There will probably be more nanotechnology, microchips, and biosensor systems that can perform tests "faster and faster and cheaper and cheaper," Dr. Fung says. And robotics will definitely play a big role.

More research will help a new generation of microbiologists develop new and exciting rapid testing tools, Dr. Fung says. "It is important for all players in this field to stay positive and work on the issues with patience, openness, and optimism."

DEMAND DRIVES GROWTH

Driving this worldwide development and growth is an increase in food consumption, consumer demand, industry food safety priorities, and regulation, Weschler says. The acceleration of the conversion of traditional microbiological testing methods to rapid methods is a function of those phenomena. It's no surprise that, despite the higher cost per test, these newer methods are being used more frequently; compared with tra-

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ditional testing, they provide faster results and/or ease of use benefits.

Nonetheless, traditional methods still account for approximately 58% of the microbiology tests performed worldwide in the food market. Their rapid counterparts—including convenience-based, immunoassay-based, and molecular-based methods—account for 42%. In fact, over the last three years, food micro tests utilizing rapid methods have increased by 37% to 307 million tests, up from 224 million in 2005. Some 80% or more of all tests are run to determine non-pathogens or indicator organisms.

"By 2013, much will have changed," says Weschler. "Traditional methods will still be the predominant ones used, accounting for 491.2 million tests. However, traditional will represent only 50.7% of all tests conducted, which is approximately an 8% decrease based on percentage of tests performed."

All types of rapid methods will make significant gains in usage during the coming five-year period, Weschler adds. "When combined, the annual test volume of rapid methods will increase by over 55% from current levels and reach 478 million tests in 2013. The gain in market value for rapid methods will be even more pronounced than the testing volume increases, since the rapid methods have much higher average prices per test than traditional methods."

ROADBLOCKS TO PROGRESS

Currently, progress with raw material testing is limited because of the required enrichment step, the time required to grow pathogens to detectable levels. In most enrichment steps, it typically takes 10 to 24 hours or longer to identify pathogens. Ideally, pathogens should be identified in eight hours or less; in a perfect world, the task could be slashed to minutes, even seconds.

Cost is another limiting factor with some rapid test products, according to J. Stan Bailey, PhD, MS, who spent 34 years as a research food microbiologist with the U.S. Departments of Agriculture's (USDA) Agricultural Research Service. Dr. Bailey joined bioMerieux, Inc. in January as director of scientific affairs for industry. For 25 of the last 28 years, he has served on the faculty of Dr. Fung's annual two-week rapid testing methods workshop.

"At first glance, some rapid and automated kits may seem expensive, as much as $10 to $15 per test, but you have to be mindful of the total costs of a microbiological test," Dr. Bailey says. "Total costs of a conventional test include the costs of media and disposable plastics as well as the labor required for media preparation and reading and recording the results."

Other factors drive the use of commercial test kits, Dr. Bailey says. These include better quality control, consistency of results from one sample batch to the next, time savings, and International Organization for Standardization or Association of Analytical Communities certification and documentation.

Sample handling has a major impact on the success of any test, rapid or conventional, Dr. Bailey says. Much work is being done to improve sample handling, he points out, including advances in concentration techniques, which improve the sensitivity of pathogen assays.

"For the past 20 years we've seen a pretty strong movement toward the use of automation to detect pathogens and indicator organisms," Dr. Bailey says. "Almost all laboratories are being asked to do more with less resources, and the best solution to this pressure is better automation. One of our biggest needs is a rapid test to detect and count yeasts and molds in order to determine shelf life and spoilage potential."

Automation of data will be a driver in the years ahead, Dr. Bailey adds. "We also need better connectivity so that for both quality indicators and pathogens we can automatically transfer laboratory results into data management systems," he says. "We need a data management system that will allow companies to monitor the microbiology results from all their locations, even companies in different countries, in real time."

NEW TECHNOLOGIES

Arun Bhunia, BVSc, PhD, professor of molecular food microbiology at Purdue University, focuses much of his research on the development and application of optical biosensors—including light scattering, fiber optic, surface plasmon resonance (SPR), and cell-based—all of which are used for pathogen and toxin detection.

As we push the boundaries of detection threshold, the sensor device becomes more susceptible to interferences from food matrices, background resident microflora, and inhibitors. In addition, bacterial physiology and genetic regulation of antigens that are essential for immune sensor-based detection should be well understood.

—Arun Bhunia, BVSc, PhD, Purdue University

A light scattering sensor is a laser-based entity. Shining this sensor on a bacterial colony growing on an agar plate enables researchers to record a scattered image. *(Continued on p. 20)*

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"That image is a fingerprint unique to a particular organism," Dr. Bhunia says. "Without needing any DNA, reagents, or antibodies, we can identify a pathogen by comparing the fingerprint to those stored in a huge database. We already have fingerprints for many species and strains of *E. coli*, *Listeria*, *Salmonella*, *Vibrio*, and *Staphylococcus*."

The time to identification depends on the amount of time needed for the colony to grow, as much as 12 to 15 hours for *E. coli* O157:H7, *Salmonella*, and *Vibrio*, and 24 to 30 hours for *Listeria*. "The benefit of light scattering is that organisms can be identified immediately, once colonies are available," Dr. Bhunia says. This promises to be an improvement over the two to three hours now needed for PCR results.

Dr. Bhunia and his collaborators, a group that includes Purdue engineers, have built two such prototype instruments, which they call BARDOT (BActerial Rapid Detection using Optical light scattering Technology). One will soon be provided to the USDA for further testing. "We are exploring the possibility of commercializing this instrument," Dr. Bhunia says. "It holds promise for identifying pathogens in raw and cooked product."

Measuring the interaction between two molecules, SPR works when antibody is placed on a sensor and bacteria or toxin are added. "The pathogen will react with the antibody and change the angle of reflectance, which can then be measured," Dr. Bhunia says.

A sensor features optical fibers with different antibodies. Binding of a target pathogen to the antibody-coated fiber can be detected using a fluorescent-labeled second antibody. The resulting fluorescent signal is proportional to the amount of target agents in a sample.

Dr. Bhunia has developed an innovative mammalian cell-based sensor in a 96-well plate format that makes it possible to test a large number of samples at once using a standard plate reader. This functional diagnostic sensor allows quick analysis—one to two hours—of the virulence potential of viable pathogens or active toxins.

"Recent developments in sensor technologies appear to be promising and sound exciting," Dr. Bhunia says. "However, there are a few challenges we must continue to address to make this technology robust. As we push the boundaries of detection threshold, the sensor device becomes more susceptible to interferences from food matrices, background resident microflora, and inhibitors. In addition, bacterial physiology and genetic regulation of antigens that are essential for immune sensor-based detection should be well understood."

Dr. Bhunia adds that sensor technology must be made more affordable, portable, and capable of testing multiple organisms. "Currently, light scattering instruments and fiber optic sensors cost $35,000 to $40,000. The challenge to private companies for the next level of commercial development is to make the technology as user friendly as possible." ■

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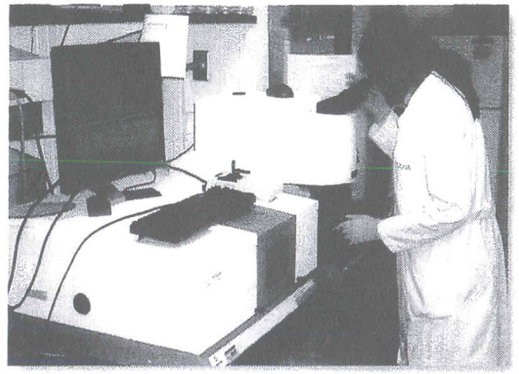
by Neil H. Mermelstein

Testing for *Salmonella*

A foodborne illness outbreak that began in April 2008 and spread over 43 states, the District of Columbia, and Canada made more than 1,430 people sick and may have led to two deaths. An intensive epidemiological investigation beginning in May by the Centers for Disease Control and Prevention in collaboration with the Food and Drug Administration, the Indian Health Service, and state public health officials identified the cause of the outbreak as the Saintpaul serotype of *Salmonella enterica* and the source of the illness as jalapeño and serrano peppers imported from Mexico. The investigation initially identified tomatoes as the source, but FDA later lifted its warning on consuming them. Updates can be found on CDC's Web site (www.cdc.gov/salmonella/saintpaul) and FDA's Web site (www.fda.gov/oc/opacom/hottopics/tomatoes.html).

One of the problems involved in correctly identifying the real culprit, according to Ahmed E. Yousef (yousef.1@osu.edu), Professor of Food Microbiology at The Ohio State University, Columbus, is that there are about 2,500 serotypes of *Salmonella* and many subtypes in each, so identifying the serotype is not enough. Simply finding the *Salmonella* serotype in a patient and a food is not 100% proof that this particular food is the cause of the disease outbreak, he said. We have to identify the specific subtype and show that the bacterium in the food is the same as that isolated from the people experiencing the illness. Isolates of the Saintpaul serotype have genetic variations, he said, so to link the food to the illness, we need to match the genetic fingerprint of *Salmonella* Saintpaul from both sources.



***OSU graduate student** Veena Prabhakar uses a Varian FTIR microscope to "fingerprint" a* Salmonella *isolate.*
Photos by Anand Subramanian, Ohio State University

*We have to **identify the specific subtype and show that the bacterium in the food is the same as that isolated from the people** experiencing the illness.*

Techniques for Identification

Conventional microbiological methods used to detect and identify *Salmonella* serotypes may require up to 5–6 days, so rapid and automated methods based on serological and molecular approaches have been developed.

A fingerprinting technique called pulsed-field gel electrophoresis (PFGE) is CDC's method of choice for epidemiologic subtyping of pathogenic bacteria. It produces unique DNA patterns that serve as bacterial fingerprints. CDC developed standardized PFGE methods and in collaboration with the Association of Public Health Laboratories (APHL) created a national database called *PulseNet* so that scientists at public health laboratories throughout the country could rapidly compare the fingerprint patterns of bacteria isolated from ill persons to those in the database to identify specific bacteria and aid in epidemiological investigations.

Laboratories participating in *PulseNet* perform DNA fingerprinting by PFGE on disease-causing bacteria isolated from humans and from suspected food using standardized equipment and methods. DNA from isolated bacteria is fragmented, and the resulting fragmentation patterns are compared. Non-conventional electrophoretic equipment facilitates the migration of large DNA fragments

Testing for *Salmonella* continued...

through agarose gels by constantly changing the direction of the electrical field during electrophoresis.

Yousef said that even though PFGE is a very reliable test, slight variations in the protocol can produce different patterns. Some irregularities in fragmentation patterns can result even if everyone follows the protocol agreed on. The PFGE protocol is also time-consuming, having a turnaround time of about five days. For these reasons, Yousef and Luis Rodriguez-Saona (rodriguez-saona.1@osu.edu), Assistant Professor in OSU's Dept. of Food Science and Technology, have been developing a rapid, high-throughput technique for detecting and differentiating *Salmonella* serotypes and subtypes. The technique—Fourier transform infrared (FTIR) microspectroscopy—is said to be easier, more reliable, and much faster than the PFGE technique. It seems to be less affected by variations in protocol, Yousef said, so it may be better than PFGE for interlaboratory comparisons.

In FTIR analysis, the absorption of infrared radiation by the bacteria produces a pattern of bands—a spectrum—that is unique to the strain, which might be a serotype or a subtype of *S. enterica*. Rodriguez described the FTIR procedure as follows: A cell suspension of the test bacterium is filtered through a hydrophobic-grid membrane filter (HGMF). The filter is then incubated on a suitable agar medium for up to 24 hr and dried before being examined by an IR microscope. A germanium-tipped probe attached to the microscope produces a wave that interacts with bacterial cells on the filter and produces a spectrum. The spectra collected are subjected to principal-component statistical analysis, and statistical models called soft independent modeling of class analogy (SIMCA) models are developed. Analysis of the filter-mounted cells takes less than 15 min.

The limiting factor, Rodriguez said, is growing enough of the bacteria on the hydrophobic grid to reach a specific biomass (104–105 cells) that would give a reproducible signal. This usually takes 12–18 hr. Also, the growth media will influence the spectral fingerprint, and use of selective media has improved the differentiation among different isolates of *S. enterica* serotypes. The HGMFs are dried before examination because water produces a very high signal in IR that would mask the signal from the bacteria.

Rodriguez said that analysis of the spectra has shown well-defined clusters for each *Salmonella* serotype and differentiation among serotypes, including Saintpaul. He added that multivariate analysis has shown that lipopolysaccharides in the bacterial cell outer membrane are responsible for the differentiation among isolates.

IR technology is growing at a fast pace, he said. Various companies offer benchtop equipment for the technique, including Varian (www.varianinc.com), Thermo Scientific (www.thermo.com), Bruker Optics (www.brukeroptics.com), Shimadzu (www.shimadzu.com), and Jasco (www.jascoinc.com). Smiths Detection (www.smithsdetection.com) makes a one-piece IR microscope that transforms a commercial light microscope into a versatile infrared microprobe for molecular analysis. Rodriguez added that portable handheld infrared spectrometers equipped with an attenuated total reflectance (ATR) diamond crystal (www.ahurascientific.com) are becoming available and could provide direct fingerprinting strategies for rapid and specific microbial analysis.

Table 1. AOAC Performance Tested Methods℠ for* Salmonella *testing.
From www.aoac.org/testkits/testedmethods.html#microbiological.

Test Kit	Manufacturer	Web Site
Reveal	Neogen Corp.	www.neogen.com
Bioline *Salmonella* ELISA Test Kit	Bioline	www.bioline.dk
Oxoid *Salmonella* Rapid Test	Oxoid Ltd.	www.oxoid.com
Gene Trak *Salmonella* DLP Assay	Neogen Corp.	
GeneQuence *Salmonella* Microwell Assay	Neogen Corp.	
Pathatrix System for *Listeria/Salmonella* species	Matrix MicroScience Ltd.	www.matrixmsci.com
Pathatrix System for *Salmonella* species	Matrix MicroScience Ltd.	
Pathatrix *Salmonella* species Pooling Test System	Matrix MicroScience, Inc.	
Pathatrix *Salmonella* "10" Pooling	Matrix MicroScience Ltd.	
Bax® System with Automated Detection PCR Assay for Screening for *Salmonella*	Qualicon Inc.	www.qualicon.com
RapidChek *Salmonella* Assay	Strategic Diagnostics Inc.	www.sdix.com
Roche Diagnostics LightCycler® foodproof® *Salmonella* Detection Kit for *Salmonella* spp. In combination with *Salmonella* ShortPrep foodproof I Kit	Roche Diagnostics GmbH/ Biotecon Diagnostics GmbH	www.roche-applied-science.com, www.bc-diagnostics.com
Singlepath® *Salmonella* Lateral Flow Assay	Merck KGaA/EMD Chemicals Inc.	www.emdchemicals.com
Warnex™ Rapid Pathogen Detection System for *Salmonella*	Warnex Diagnostics Inc.	www.warnex.ca
BBL™ CHROMagar™ *Salmonella*	BD Diagnostics	www.bd.com/ds
Transia Plate *Salmonella* Gold	Ralsio Diagnostics AB	www.ralsiogroup.com
DuPont Lateral Flow System *Salmonella* Test Kit	DuPont Qualicon	www.qualicon.com
Assurance GDS for *Salmonella*	BioControl Systems Inc.	www.rapidmethods.com
RapidChek™ Select™ *Salmonella* Test	Strategic Diagnostic Inc.	
iQ-Check *Salmonella* II Real-Time PCR Test Kit	Bio-Rad Laboratories	www.foodscience.bio-rad.com
TaqMan® *Salmonella enterica* Detection Kit	Applied Biosystems	www.appliedbiosystems.com
R.A.P.I.D.® LT Food Security System (FSS) for *Salmonella* Detection	Idaho Technology Inc.	www.idahotech.com

What's Needed

Daniel Y.C. Fung (dfung@ksu.edu), Professor of Food Science at Kansas State University, Manhattan, said that advances in rapid detection of pathogens are occurring, but we still need to have very good statistical sampling procedures, as well as very good enrichment procedures to let *Salmonella* grow so we can detect it. We also need very good antibodies to type the serotypes. Polyclonal antibodies trap more antigens, but monoclonal antibodies are more specific of an organism's antigen. We need monoclonal antibodies specific for the Saintpaul serotype.

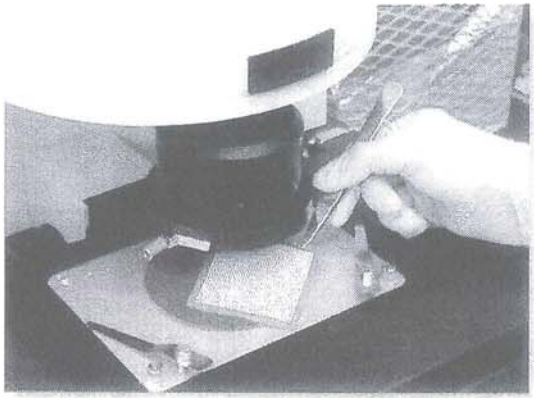
With rapid methods, if we get a presumptive negative, we're done, he said—there's no *Salmonella* present. However, if we get a presumptive positive, then we still need to go through the conventional culture method to confirm it. The gold standard is the conventional method described in FDA's *Bacterial Analytical Manual* (www.cfsan.fda.gov/~ebam/bam-toc.html); the method for *Salmonella* can be found in chapter 5 (www.cfsan.fda.gov/~ebam/bam-5.html).

Rapid test kits are very reliable, he added. If a kit can detect the organism 90–95% of the time, it's reliable. A number of test kits for *Salmonella* detection have received *Performance Tested Methods* certification from the AOAC Research Institute, which provides an independent third-party review of test kit performance claims. These methods are listed in Table 1.

Fung briefly reviewed some of the approaches to detection and identification of microorganisms:

- Monoclonal and polyclonal antibodies are used to bind with antigens on the organism's surface to detect and characterize microorganisms and their components. Some companies

Testing for *Salmonella* continued...



A hydrophobic grid membrane filter *bearing the dried bacterial culture is placed under an FTIR microscope in attenuated total reflectance mode to produce a spectrum that can be used to identify the bacterial strain.*

offer completely automated enzyme-linked immunosorbant assays (ELISAs). For example, the VIDAS system from bioMèrieux (www.biomerieux-usa.com) can automatically perform ELISAs in 45 min–2 hr after pre-enrichment of target organisms.

• Lateral-flow antigen–antibody tests, often referred to as dipstick tests, take only about 10 min after overnight enrichment.

• Immunomagnetic separation is being used to capture target molecules, reducing incubation time and increasing sensitivity. The *Pathathrix* system from Matrix MicroScience Ltd. (www.matrixmsci.com), for example, recirculates a pre-enriched sample over antibody-coated paramagnetic beads for 30 min to capture as many target organisms as possible for subsequent detection by ELISA, PCR, culture, or other methods.

• Polymerase chain reaction (PCR) detects pathogens by amplifying the target DNA and detecting the target PCR products. The target products have conventionally been detected by gel electrophoresis, which is time consuming, but faster fluorescence techniques are now on the market (real-time PCR). For these rapid techniques, the more target DNA in the sample, the faster the results will be. For example, the *Salmonella* detection kit from Applied Biosystems (www.appliedbiosystems.com) uses real-time PCR to identify pathogens in food in less than 24 hr.

Fung has been conducting for the past 28 years Kansas State's annual International Workshop/Symposium on Rapid Methods and Automation in Microbiology. At these eight-day workshops, attendees hear experts discussing the newest advances and participate in hands-on sessions with new equipment, techniques, and test kits without making commitments to purchase the equipment and systems. More than 4,000 scientists from 60 countries have participated in these workshops. The most recent workshop, held June 13–20, 2008, also featured a special day-long session showcasing presentations on cutting-edge developments in molecular biology. The next workshop will be held in Manhattan, Kan., on June 19–26, 2009. **FT**

MÉTODOS RÁPIDOS Y AUTOMATIZACIÓN EN MICROBIOLOGÍA ALIMENTARIA

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El pasado noviembre, tuve la oportunidad de asistir al Workshop sobre Métodos Rápidos y Automatización en Microbiología Alimentaria que, bajo la dirección de los doctores Marta Capellas Puig y Josep Yuste Puigvert, se celebra anualmente en la Facultad de Veterinaria de la Universidad Autónoma de Barcelona. Se trata de un curso muy interesante que permite adquirir una amplia visión de los métodos de detección e identificación rápida de los microorganismos presentes en los alimentos, así como conocer los últimos avances aplicables al análisis microbiológico en la industria alimentaria. El curso consta de: (i) conferencias impartidas por destacados científicos españoles y extranjeros; (ii) presentaciones a cargo de expertos de importantes empresas de microbiología, incluyendo "rotaciones" en las que grupos reducidos de participantes en el curso asisten a la demostración del funcionamiento de equipos y productos; y (iii) dos sesiones prácticas de laboratorio, un taller y una visita a una empresa de Biología Molecular. Entre los conferenciantes que intervinieron en el curso, destaca la figura del Dr. Daniel Y. C. Fung, profesor de la Kansas State University en Manhattan (Kansas) y director del mundialmente conocido Workshop on Rapid Methods and Automation in Microbiology que se celebra anualmente en la mencionada universidad desde 1980. El Dr. Fung, autoridad internacional en el campo de los Métodos Rápidos en Microbiología y autor de más de 800 artículos científicos en la materia, impartió seis interesantes conferencias basadas en su artículo *Rapid Methods and Automation in Microbiology* (1), cuyos aspectos más destacados presento a continuación.

El desarrollo de métodos rápidos y automatizados para la detección, aislamiento, identificación y enumeración de microorganismos (y/o sus metabolitos) relacionados con la alteración y seguridad de los alimentos es una subdivisión del área de la microbiología aplicada con una importancia cada vez mayor. En este sentido, según investigaciones de mercado realizadas en 2003 por Strategic Consulting Inc.'s, del total de ensayos microbiológicos realizados por la industria en el mundo (1.136,5 millones), el 58% (660, 5 millones) correspondieron a la industria de la alimentación (49%) y bebidas (9%). Aproximadamente, el 20% de los análisis se dirigieron a la detección de los microorganismos patógenos *Salmonella* y *Escherichia* coli O157:H7 y el 80% restante fueron análisis rutinarios (recuento total, de coliformes, de mohos y de levaduras). A nivel mundial, el porcentaje de ensayos realizado en Europa, América del Norte y el resto del mundo fue de un 33% en cada uno de los casos. No obstante, se estima que en un futuro próximo el porcentaje de ensayos realizados en el resto del mundo podría incrementarse hasta el 50% debido a la concienciación que están experimentando países con economías en desarrollo sobre la gran importancia de la seguridad alimentaria (1, 2, 3). Así por ejemplo, las autoridades sanitarias de China, país que se ha visto envuelto en varias ocasiones en escándalos debidos a la exportación de alimentos con condiciones higiénico-sanitarias no adecuadas, están tomando las medidas necesarias para asegurar el suministro de alimentos seguros a los más de 10.000 atletas y 3 millones de espectadores que se calcula asistirán a los Juegos Olímpicos que se celebrarán en agosto de este año en Beijing (4).

Los métodos microbiológicos "convencionales", empleados actualmente en numerosos laboratorios de todo el mundo y establecidos en muchos casos como métodos estándares de análisis microbiológico de los alimentos, se caracterizan por ser laboriosos, emplear grandes volúmenes de medios de cultivo y requerir un tiempo considerable para la obtención y el análisis de los resultados. Por el contrario, los métodos rápidos requieren un tiempo reducido para la obtención de los resultados y/o permiten procesar un número elevado de muestras por unidad de tiempo, y son en general fáciles de usar, precisos (sensibilidad y especificidad adecuadas y límites de detección bajos) y económicamente rentables (aunque, en algunos casos, pueden requerir una inversión económica inicial considerable). Conviene destacar que, en la mayoría de los casos, el empleo de métodos rápidos no excluye la etapa de enriquecimiento del microorganismo diana ni la necesidad de obtener cultivos puros, así como que los resultados positivos obtenidos con métodos alternativos (distintos del método de referencia) deben ser confirmados. Asimismo, es de suma importancia, al igual que en el caso de los métodos tradicionales, una adecuada toma y preparación de las muestras a analizar. En lo que se refiere a este último aspecto, destacan los siguientes avances: (i) para muestras sólidas, instrumentos gravimétricos que realizan automáticamente las diluciones programadas (Dilumat, AES Chemunex; Dilumacher, PBI; Labpro Gravimetric Diluter, Spiral Biotech) y homogeneizadores que funcionan mediante ondas de choque y una intensa agitación para transferir los microorganismos del alimento al diluyente, produciendo una mínima destrucción de la muestra (Pulsifier,