2009

Sample Preparation: The Forgotten Beginning

Byron F. Brehm-Stecher
Iowa State University, byron@iastate.edu

Charles Young
Johns Hopkins University

Lee-Ann Jaykus
North Carolina State University

Mary Lou Tortorello
United States Food and Drug Administration

Sample Preparation: The Forgotten Beginning

Abstract
Advances in molecular technologies and automated instrumentation have provided many opportunities for improved detection and identification of microorganisms; however, the upstream sample preparation steps needed to apply these advances to foods have not been adequately researched or developed. Thus, the extent to which these advances have improved food microbiology has been limited. The purpose of this review is to present the current state of sample preparation, to identify knowledge gaps and opportunities for improvement, and to recognize the need to support greater research and development efforts on preparative methods in food microbiology. The discussion focuses on the need to push technological developments toward methods that do not rely on enrichment culture. Among the four functional components of microbiological analysis (i.e., sampling, separation, concentration, detection), the separation and concentration components need to be researched more extensively to achieve rapid, direct, and quantitative methods. The usefulness of borrowing concepts of separation and concentration from other disciplines and the need to regard the microorganism as a physicochemical analyte that may be directly extracted from the food matrix are discussed. The development of next-generation systems that holistically integrate sample preparation with rapid, automated detection will require interdisciplinary collaboration and substantially increased funding.

Disciplines
Food Science | Human and Clinical Nutrition

Comments
This article is from *Journal of Food Protection* 72, no. 8 (2009): 1774–1789.

Journal of Food Protection, Vol. 72, No. 8, 2009, Pages 1774–1789

Review

Sample Preparation: The Forgotten Beginning

BYRON BREHM-STECHER,[1] CHARLES YOUNG,[2] LEE-ANN JAYKUS,[3] AND MARY LOU TORTORELLO[4]*

[1]Department of Food Science and Human Nutrition, Iowa State University, Ames, Iowa 50011; [2]National Security and Technology Department, The Johns Hopkins University Applied Physics Laboratory, 11100 Johns Hopkins Road, Laurel, Maryland 20723; [3]Department of Food, Bioprocessing, and Nutrition Sciences, North Carolina State University, Raleigh, North Carolina 27695-7624; and [4]U.S. Food and Drug Administration, National Center for Food Safety and Technology, 6502 South Archer Road, Summit-Argo, Illinois 60501, USA

MS 09-034: Received 22 January 2009/Accepted 14 March 2009

ABSTRACT

Advances in molecular technologies and automated instrumentation have provided many opportunities for improved detection and identification of microorganisms; however, the upstream sample preparation steps needed to apply these advances to foods have not been adequately researched or developed. Thus, the extent to which these advances have improved food microbiology has been limited. The purpose of this review is to present the current state of sample preparation, to identify knowledge gaps and opportunities for improvement, and to recognize the need to support greater research and development efforts on preparative methods in food microbiology. The discussion focuses on the need to push technological developments toward methods that do not rely on enrichment culture. Among the four functional components of microbiological analysis (i.e., sampling, separation, concentration, detection), the separation and concentration components need to be researched more extensively to achieve rapid, direct, and quantitative methods. The usefulness of borrowing concepts of separation and concentration from other disciplines and the need to regard the microorganism as a physicochemical analyte that may be directly extracted from the food matrix are discussed. The development of next-generation systems that holistically integrate sample preparation with rapid, automated detection will require interdisciplinary collaboration and substantially increased funding.

A great deal of effort is spent on improving microbiological methods. Each year, dozens of articles are published, and hundreds of presentations are made at scientific meetings in the category of ''microbiological methods research.'' News items and press releases routinely herald developments of methods that promise more rapid and specific detection of microorganisms. These efforts are highly commendable, given the difficulty, length of time, and expense associated with detecting and differentiating microorganisms.

Much of the research efforts, however, involve only the final portion of the procedure, i.e., the detection or identification of the microorganisms. The ''upstream'' portions that deal with sampling and sample preparation are often overlooked. It is easy to understand why this is so. The detection and identification aspects appeal to what is naturally interesting to microbiologists, i.e., the intrinsic characteristics of the microorganisms, which can be exploited to differentiate one from another in an analytical method. The unique metabolic traits, structural components, antigenic constituents, or nucleic acid sequences comprise the cellular targets around which novel and very specific detection or identification methods may be designed. The huge advances in genomics and molecular microbiology are uncovering ways to very specifically distinguish one microorganism from another, and they offer many opportunities for novel application in methods research.

* Author for correspondence. Tel: 708-728-4146; Fax: 708-728-4177; E-mail: mary.tortorello@fda.hhs.gov.

Despite these efforts and advances, the food microbiologist is often disappointed to find that the novel detection technologies have not been developed with the food matrix in mind. The upstream sample preparation steps necessary for working foods into the novel assays have not been considered. In general, novel analytical technologies are often developed for clinical microbiology applications—understandable, perhaps, due to the lucrative nature of the clinical diagnostics market. In foods, the combination of low levels of contamination and the complexity and diversity of sample matrices provides challenges when trying to adapt novel molecular-based detection technologies outside of the clinical realm. Biodefense and environmental interests have served to broaden applications beyond the clinical focus by supporting research in improved testing of air, water, and environmental surfaces, but applications of the novel technologies to foods are still relatively uncommon.

Ideally, methods for preanalytical sample preparation of foods should accomplish one or all of the following functions: separate target cells from the food, increase their concentration, purify them from extraneous material and nontarget biota, achieve volume reduction in bulk samples, produce a homogeneous sample, and exclude inhibitory substances. Unfortunately, a food matrix is a tough challenge, which limits or defeats many novel method applications. Foods are messy and nearly unlimited in variety, so that a ''catch-all'' preparative method seems virtually impossible to develop. Also, despite our best efforts to prepare the matrix for detection, residual, food-associated

compounds frequently interfere with the detection assay. So, although novel detection technologies will continue to be sought to improve the efficiency of analysis, for the food microbiologist, it is the upstream portions of the procedure, i.e., those involving sampling and food sample preparation, which need the greatest research attention now.

Novel technologies aside, a closer look needs to be taken at upstream procedures, even as they are applied to traditional detection technologies. There is inconsistency, if not guesswork, in the ways in which various food samples are prepared for standard (cultural) microbiological analysis. While the detection and identification procedures are generally straightforward and well described, preparative methods are sometimes based on best judgment or personal experience rather than well-validated scientific study.

In this article, we discuss the current state of sample preparation and the hurdles to overcome in achieving more rapid microbiological analysis of foods. Although the inconsistency and lack of standardization in preparative methods for traditional, enrichment-based analysis is recognized as an important issue, this discussion focuses on the need to push technological developments toward nonenrichment analysis for speed, quantification, and applications in which enrichment may not be possible. It is hoped that an outgrowth of this discussion will be greater recognition of the knowledge gaps and of the need to support research on preparative methods for food microbiology.

THE WAY IT IS

Microbiological methods may be classified by the type of data generated, i.e., qualitative or quantitative, and a sample preparation method may need to be chosen depending on the type of data needed. Qualitative assays are designed to detect and identify the presence of a particular microorganism or microbial group. Quantitative assays provide estimates of the population of a microorganism or group of microorganisms in a sample. Virtually all the pathogen detection methods available in food microbiology are qualitative (presence or absence) rather than quantitative.

Qualitative assays generally include the technique of enrichment culture as a starting point. Enrichment culture involves adding the food (after some type of preparative step) to an appropriate nutritional medium and incubating for many hours to allow the target microbial cells to grow. Enrichment culture conditions are designed to meet the specific growth requirements of the target organism(s) while inhibiting, to the greatest extent possible, the growth of other microorganisms. Thus, the target population, which is often present at low levels relative to the ''background'' microorganisms in the food, is amplified relative to the background microbiota. Enrichment culture may also help to revive microbial cells that may have been stressed or injured by exposure to chemical or physical treatments used in food production or processing. Typically, in the enrichment procedure, a 1:10 dilution of the food matrix is made (e.g., 25 g of food in 225 ml of enrichment medium); thus, interferences from the food in the detection assay may be reduced. Enrichment culture is an extremely sensitive technique, because it can promote the amplification of a single cell to levels $\geq 10^3$ CFU/ml of enrichment broth, at which point detection is possible.

Although enrichment culture techniques are the standard means by which to deliver the food matrix to a detection assay, they are problematic in certain respects: they are lengthy and compromise rapid analysis; they are not available for certain important targets, e.g., viruses, parasites, fastidious bacterial pathogens; they may fail to detect certain targets, e.g., sublethally injured microorganisms, if the enrichment conditions are not permissive to their growth; and they do not allow for enumeration of the target microbe in the sample, a notable exception being the combination of enrichment culture with the most-probable-number technique. As generally used, enrichment culture effectively ''erases'' valuable information about initial microbial numbers within a sample, downgrading a potentially quantitative test into a qualitative one.

Enrichment methods have served us well for decades; however, as food microbiology enters the 21st century, there is a need for enumerative data on pathogen contamination. For example, enumeration is necessary to achieve improved understanding of the kinetics of microbial growth or inactivation, to assess microbial behavior in foods and processing environments for devising effective controls, to estimate microbial populations for surveillance purposes, and to inform quantitative risk assessments. While previously considered very difficult to obtain, i.e., by most-probable-number analysis, enumerative data for specific pathogens in foods may become easier to generate by using more rapid quantitative molecular assays, such as quantitative real-time PCR.

THE WAY IT COULD BE

It should be clear from the previous discussion that reduction of total assay time with production of enumerative results would be the ideal scenario for foodborne pathogen detection. Although many rapid assays have been introduced that use a variety of detection platforms (including PCR), they are still generally qualitative and rely on a time-consuming enrichment step to increase population levels to reach the lower limits of detection of the assays. If time is not an issue, then enrichment provides a simple solution to the problem of obtaining sufficient target for the assay; however, given the speed and capacity of global food production and distribution networks, time is *the* limiting factor, and analytical shortcuts are critically needed to take us rapidly from the initial food sample to detection.

Nonetheless, assay developers still focus on improving the detection component of the assay, and continue to rely on the availability of enriched samples. Our thesis is that the development of rapid detection methods *in isolation* is inadequate if we are to eventually move the field of foodborne pathogen detection forward. The fact remains that detection is a final downstream event that has little intrinsic value if it is not closely integrated with critical upstream inputs such as effective separation of target cells from the food matrix and their subsequent concentration to a suitable volume. The dilemma would be solved by developing efficient non–enrichment-reliant methods to separate targets

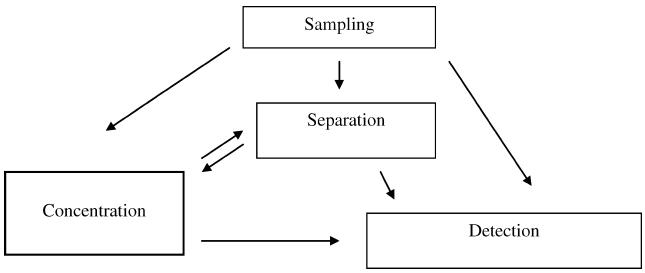

FIGURE 1. *Functional components of food microbiological analysis.*

from the food matrix and concentrate them to sufficient levels for delivery to the assay, which ideally would produce quantitative data.

A HOLISTIC VIEW OF FUNCTIONAL COMPONENTS

Regardless of whether qualitative or quantitative outputs are desired, there are four functional components of any method: sampling, separation, concentration, and detection. The relationship of these functional components to each other is shown in Figure 1.

Sampling. Sampling, which involves considerations of unit size (e.g., volume, mass, number) is usually dictated by the purpose of the analysis (e.g., lot acceptance, screening, surveillance, outbreak investigation) and some knowledge of the microbial load and distribution of the target in the sample. Practical constraints of time and cost are also a factor. The International Commission on Microbiological Specifications for Foods has written extensively on sampling and the development of sampling plans for food products *(29, 30).* Information addressing specific sampling proposals for various commodities, as well as a spreadsheet tool for calculating acceptance probabilities for foods having different bacterial loads, have been made available as free downloads from the Internet (http://www.icmsf.iit.edu/main/home.html). Sampling, therefore, is not discussed further in this article, although its importance in the design and usefulness of any detection assay is critical.

Detection. How best to arrive at detection from sampling is the pursuit of all methods researchers. In an ideal world, a food could be analyzed directly, in real time, with detection of microorganisms immediately after sampling. Electronic nose and tongue technologies are continually improving, yet reliable ''sniff and find'' or ''taste testing'' of foods for the presence of microbes are goals that are still far from practical realization *(4, 43, 51, 64, 78).* Current detection assays use a variety of formats and instrumentation, beyond the scope of this discussion.

Concentration. Because target microorganisms may be present at very low levels, and it is necessary to process large volumes (≥25 g, and as much as 375 g in some cases), concentration is an essential functional component. In other words, there is a need for some means by which to increase the number of targets into a volume compatible with the chosen detection assay. This ''concentration'' currently is achieved by target amplification methods: traditionally by enrichment culture (which involves amplification of cell numbers), but also by PCR (nucleic acid amplification) and some immunoassays (ligand binding or signal amplification). During enrichment, a food microbe multiplies from as few as a single cell to millions or billions of cells, while the volume of the enrichment medium does not change. This is effectively a multiplication-based concentration of the initial inoculum, as the sample volume remains the same.

Nucleic acid amplification methods such as PCR are capable of enriching a single specific DNA or RNA sequence up to a million-fold in a few hours and provide a detection limit as low as a single copy of the nucleic acid target. A primary advantage of this technology as applied to the detection of foodborne pathogens is the theoretical potential to replace cultural enrichment with specific nucleic acid sequence enrichment, thereby substantially decreasing time to detection. Furthermore, these methods have high specificity of detection and can facilitate the identification of microorganisms that are difficult or impossible to culture, two additional features of interest to the food microbiologist. Unfortunately, sample volume constraints (PCR uses volumes of a few microliters rather than milliliter volumes) are an important limitation of PCR and as the technology is currently marketed, can only be overcome with prior cultural enrichment.

Separation. Concentration usually goes hand in hand with perhaps the functional component most overlooked in food microbiology: separation, or the removal of food matrix components that might interfere with detection. Separation constraints are also the factor that most often prevents the application of very rapid methods. Food-associated inhibitory substances are often recalcitrant to removal steps and frequently inhibit the activity of the enzymes used in nucleic acid amplification, as well as reagents of immunoassays and biosensor components. Enrichment culture may help to reduce interference from particulates or biochemical inhibitors, simply through dilution *(20),* but generally, additional dilution steps or separation techniques are still needed to remove inhibitors present in the enrichment culture sample.

If our ultimate goal is to reduce time to detection, eliminate the need for cultural enrichment, and provide quantitative data on pathogen load, the techniques chosen for preanalytical sample processing must adequately address the separation and concentration issues that plague environmental and food microbiologists: concentration to achieve sample-size reduction and target amplification, and separation of pathogens and removal of inhibitory compounds associated with the matrix. Benefits of such separation-concentration techniques include not only increased sensitivity (lower detection threshold), but also earlier and easier detection *(74).* An apt analogy would be comparing the ease and efficiency of shooting fish in a barrel of clear water versus shooting the same number of fish in a murky lake. It is clear that extraction, removal, and/or separation of cells from the foods in which they are dispersed, or to which

they are attached, is the major technological hurdle that must be addressed in methods research. Various categories of separation methods and their underlying principles are compared in Table 1.

AS OLD AS MODERN MICROBIOLOGY

Although the importance of sample preparation to successful detection of microbial pathogens in foods is undergoing a period of renewed recognition, the need for methods capable of separating and concentrating cells from sample matrices is as old as modern microbiology. Early examples (1905 to 1930) include descriptions of chemical precipitation with alum or combinations of sodium hyposulfite and lead nitrate for concentration of *Bacillus typhosus* from large volumes of water *(92)*; electrophoretic concentration of bacteria, including *Staphylococcus aureus* and *Bacillus coli* from liquid suspensions or urine *(71)*; and a method for ''baiting'' acid-fast bacteria with paraffin as a means of separating them from buffer-based soil suspensions *(22).* The remarkable timelessness of these early approaches attests not only to the ingenuity of these early investigators, but also to the basic and lasting nature of the problem: the underlying need to first separate and concentrate microbes from sample matrices so that we may then detect them. Unlike these early researchers, we have an almost cornucopic abundance of high-purity chemicals, novel materials or components, and off-the-shelf processing instrumentation at our disposal. There has never been a better time for addressing (and hopefully solving) the basic problems of preanalytical food sample preparation.

SEPARATION AND CONCENTRATION METHODS

Methods of separation and concentration of microorganisms from foods can be categorized as physical, chemical, adsorptive, bioaffinity-based, etc. Many methods actually apply any number of these general principles in combination *(7, 80).* Furthermore, separation and concentration schemes can be used singly or in combination, but in all cases, the goal is to provide a sample of extremely small volume with high recovery of the target pathogen and removal of inhibitory compounds. Some of the more commonly used separation principles are described below briefly.

Physical separation. Centrifugation and filtration offer effective primary means to separate pathogens from foods. Although useful and available in ''souped-up'' versions (e.g., continuous centrifugation versus batch, tangential flow filtration versus ''dead end''), they are cumbersome, often do not provide adequate separation alone, and must be followed by other more refined methods.

Adsorptive processes. Metal hydroxides, resins, and lectins have been used to capture bacteria so that they can be separated from foods. These methods can be quite effective in reducing sample volume and removing inhibitors, but their performance differs with both target pathogen and sample matrix.

Bioaffinity separation. Biological reagents may be used to separate targets from food matrices by virtue of their specific recognition capabilities. The coupling of antibodies to magnetic beads in immunomagnetic separation was recognized two decades ago *(76)* as a way to both separate and concentrate specific bacterial cells from a food matrix. Bacteriophages have also been used as specific separators *(6).* Although initially applied to relatively small sample volumes (1 to 10 ml), recent advances are facilitating application to more realistic sample sizes of ≥25 g and/or the use of ligands other than antibodies (e.g., aptamers or phage). These methods have the advantage of high specificity and binding avidity, but they are usually specific to a single bacterial species (and sometimes a specific serovar, as, for example, in the case of *Escherichia coli* O157:H7). The use of immunomagnetic separation in the 2006 spinach-associated outbreak of *E. coli* O157:H7 infections in the United States vastly assisted the investigation and speed of resolving that public health emergency.

Additional tools. The various methods available for separation and concentration of bacterial cells from foods have been comprehensively reviewed elsewhere *(7, 80).* Table 1 summarizes these methods by general category, their principles of operation, specific examples of each, and their relative advantages or disadvantages.

Physical and biochemical challenges of the food matrix. The sheer variety of foods that make up the human diet presents unique challenges to the successful separation and concentration of cells for downstream detection. Even a seemingly simple commodity category, such as ''vegetables'' comprises not only different plant species, but also different physical preparations: sliced, shredded, fresh, fermented, etc. The range and diversity of surfaces available for potential microbial colonization is dizzying enough for fresh vegetables; however, the creation of additional surface area, decompartmentalization of tissues or physical inoculation of tissue interiors that accompanies processing, further complicates the physical environment from which we seek to extract target microbes. Adding further to the complexity of finished foods is that most contain multiple ingredients, from mixed commodity groups (e.g., a spiced chicken sandwich with vegetables and cheese), with each ingredient contributing unique physical properties and endogenous microbiota. The challenges to adequate cell separations from such environments are many and include particulate matter, fats, biochemical or inorganic food components, and the presence of nontarget microbiota. For example, particulates can foul filters or coprecipitate with target cells, serve as microniches from which cells are hard to extricate, interfere with visual or optical analyses, and/or nonspecifically bind and sequester probes or stains. The fat–water balance of a food can affect partitioning of the cells within the matrix, and many food ingredients, including fats, proteins, divalent cations, and phenolic compounds, can act as PCR inhibitors. Nontarget microbiota, especially when present in high concentrations, cause additional difficulties for the food microbiologist interested in detecting and quantifying comparatively low numbers of target cells. Additional intrinsic physical factors complicating the microbial analysis of foods include viscosity (prob-

TABLE 1. *Approaches to separation of microbial cells from complex matrices*

Method	Principles	Examples	Advantages	Disadvantages	References
Physical separation	Physical manipulation of cells via applied forces (centripetal/centrifugal, gravity, sonic, liquid flow) and interaction with surfaces (filter, test tube, air bubble surfaces) Adhesive removal of cells from food surfaces	Centrifugation (simple, differential, density gradient, continuous flow) Coagulation/flocculation/precipitation/sedimentation Filtration (dead end, tangential flow, hollow fiber) Vacuum sampling of surfaces Microfloatation using bubbles/foam Ultrasonic focusing	Can be simple, applicable to complex matrices May be used to concentrate cells through volume reduction May be used to detach cells/biofilms from surfaces or crevices May facilitate sampling of large surface areas Can be used to concentrate cells from very large volumes at smaller liquid-solid or liquid-air interfaces	Nonspecific: may concentrate target cells, nontarget flora, and particulate matter Simple centrifugation is volume limited Filters may be fouled by food particles, other components of matrix, adsorptive loss of cells to filter surfaces may limit cell recovery Some equipment may be expensive	*9, 11, 23, 32, 35, 40, 44, 81, 82, 92, 93*
Chemical separation	Partitioning of cells between two immiscible liquid (nontoxic polymer) phases. Cells accumulate at interface or preferentially into one bulk phase, depending on cell surface properties Chemical digestion of food matrix Direct extraction of diagnostic (and other) cellular macromolecules from matrix	Aqueous two-phase partitioning Matrix lysis system (buffer-solvent-detergent-chelator mixtures) SFE[a]	Simple, rapid, inexpensive SFE may allow bypass of cell separation/concentration steps through direct extraction of target molecules (nucleic acids, fatty acids, etc.) from matrix	Variable efficiency Nonspecific, nontarget cells and cell-like debris may also partition Harsh matrix lysis systems may affect cell viability, surface properties Bulk chemical extraction yields complex mixture of cellular macromolecules	*41, 49, 68, 81, 91*
Adsorptive processes	Adsorptive attachment via charge-based interactions of surface-displayed macromolecules with stationary or mobile solid phase (beads, fibers, particles)	Ion exchange/hydrophobic interaction chromatography Adsorption onto metal hydroxide particles	Can be simple, inexpensive, applicable to complex matrices (foods, feces) Can be combined with other approaches (simple centrifugation, magnetic concentration) Particles may present large surface area for interaction Physical exclusion of food particulates from pore space may provide filterlike properties	Sample must be fluidized Nontarget flora or matrix components may compete for adsorption Cells contained within food matrix microenvironments may be unavailable for adsorptive interactions	*8, 40, 45, 53, 72, 96*

TABLE 1. *Continued*

Method	Principles	Examples	Advantages	Disadvantages	References
Bioaffinity separation	Ligand-receptor interactions Based on natural biological recognition phenomena Synthetic (biomimetic or bioengineered) binders	Antibodies, carbohydrate-binding proteins (lectins), natural receptors (mucins), supramolecular binders (bacteriophage), recombinant phage binding proteins, combinatorially produced protein or peptide binders, antibiotics (vancomycin), antimicrobial peptides, synthetic glycopolymers, aptamers, molecular imprinting	Potential for specific, high-affinity binding Genetic modification of phage (LUX, ice nucleation genes, etc.) Sustainable production of additional reagents via biological processes (i.e., phage life cycle, recombinant expression, combinatorial synthesis) Synthetic binders may be intrinsically stable, both on the shelf and in foods	Specificity (exclusivity/inclusivity) variable Receptor may not be expressed by target cell in the food environment or under conditions of enrichment Biological materials subject to degradation (denaturation, enzymatic digestion, etc.)	*2, 38, 46, 52, 54, 57, 61, 65, 66, 75, 80, 83, 94*
Electrokinetic/ electrophysical manipulation	Attractive or repulsive forces exerted on whole-cell dipole in uniform (CE) or nonuniform (DEP) electric fields[b]	DEP DEP field-flow fractionation CE	Can separate cells and particles on basis of size, shape, or charge Can be used to concentrate cells at electrode surfaces or within capillaries for detection Can differentiate between viable and nonviable cells according to dielectrophoretic properties (i.e., membrane conductivity) Can be combined with whole-cell methods for molecular detection	Microscale technologies: small sample sizes may affect limit of detection May be unable to discriminate between physiologically similar cell types (although this can be addressed using cell-specific antibodies or molecular probes)	*14, 39, 42, 71, 95*
Combined approaches	Sequential use of multiple separation or concentration modalities (i.e., chemical fractionation, which is then followed by centrifugation)	Combined filtration, physicochemical separation, centrifugation, etc. Density gradient centrifugation Immunomagnetic separation, use of magnetic hydroxyapatite or cationic magnetic beads	Combined processes may yield results that are orders of magnitude more efficient than individual component steps	Limitations of individual processes still apply When linking several processes, inefficiencies of individual steps may lead to cell loss/diminishing returns	*23, 27, 73, 74, 80*

[a] SFE, supercritical fluid extraction.
[b] DEP, dielectrophoresis; CE, capillary electrophoresis.

lematic for pipetting, centrifugation, or filtration), heterogeneous distribution of target cells (microcolonies, ''hot spots''), the presence of biofilms or microbial aggregates, and ''inhibitors'' of optical analysis, such as autofluorescence from plant tissues.

BORROWING CONCEPTS

Although the fields of food, environmental, and clinical microbiology represent separate and specialized subdisciplines, they also share many of the same basic needs and goals, including those of obtaining a representative sample, effectively separating cells from the sample matrix, concentrating them into an analytically relevant volume, and labeling them for detection. Whether a microbiologist is analyzing pâté, sludge, or sputum, many of the same procedures are common to all three fields. As food microbiologists, we need to be aware of developments in such parallel (or even more distant) fields, and remain open to applying methods or solutions shown to be useful for solving similar sample preparation problems in other complex, nonfood matrices.

More generally speaking, it may also be useful to borrow concepts (as well as expertise) from other disciplines. Approaching or reframing a biological problem as a problem in chemistry or physics, for example, may be a useful means by which to generate novel solutions. In food chemistry or biotechnology, extraction of valuable compounds from plant or animal tissues involves several steps, from disruption of the tissues for release of the compound, to its eventual separation, concentration, and purification from the tissue lysate. Biochemical analyses may be done at each stage of sample preparation in an effort to evaluate the purity of the compound. In a similar fashion, the process of separating, concentrating, and purifying microorganisms from foods may also be framed as a problem in analytical chemistry. Simply stated, we need to view the microorganism as a physicochemical analyte. This change in thinking may be the first step toward successful appropriation of ''nonmicrobiological'' tools and techniques from other disciplines for application toward food-based analyses. By way of example, detection of foodborne viruses may be instructive. For the nonenrichable viral pathogens, detection relies on PCR (or actually, reverse transcription PCR); however, product-specific techniques for separating and concentrating viruses from the food matrix rely on treating the viruses as proteins, thereby using protein precipitation (polyethylene glycol, acid precipitation) and elution (alternation in ionic conditions and manipulation of pH) techniques to promote extraction and concentration of the viruses from the matrix. In the world of foodborne viruses, sample preparation is key and based on simple principles of chemistry.

EXAMPLES OF THINKING ''OUTSIDE THE BOX''

An excellent example of this type of interdisciplinary cross-pollination is in the application of capillary electrophoresis to whole microbial cells for the purpose of ''separating microbes in the manner of molecules'' *(3).* Another example of how we might reexamine a microbiological question through the lens of chemistry is Sharpe's proposal in 2003 *(74)* for a possible ''mass action'' effect governing the detachment of bacterial cells from food matrices. In the closed environment of a stomacher bag, Sharpe notes, bacterial detachment proceeds until cells in suspension reach an apparently limiting concentration—a ''wall'' beyond which no further detachment is observed. A possible mechanism for such an effect may involve competitive processes such as cell readsorption to food particles, which may themselves increase in concentration as a food sample is agitated. If a mass action or equilibrium-based effect is involved, it may be possible to drive the detachment ''reaction'' toward completion based on classic physicochemical interaction mechanisms.

Investigation of natural processes may provide inspiration for novel methods of cell separation from liquid foods or food slurries. An excellent example is that of bubble-mediated movement of microbes in aquatic environments *(77).* Briefly, bubbles rising through the water column come in contact with suspended microbes through a process of interception and attachment. As the bubbles continue to rise, additional cells may be collected. The bubbles burst when they reach the water's surface, ejecting the attached cells, where they may become aerosolized. The efficiency of this bubble-mediated microbial scavenging depends on a number of factors, including bubble size and motion, the surface properties of the bacterial cell, and simultaneous interceptive collection of surface-active materials at the bubble's surface. Bubble-mediated transfer can theoretically concentrate bacterial cells by factors ranging from hundreds or thousands of times their concentrations within the bulk fraction of the water *(10, 12, 18, 89).* This type of natural ''physical enrichment'' has been documented to increase virus concentration up to 50 times that in the bulk phase *(5).* Evaporative concentration and aggregative effects known to occur during aerosolization of virus particles *(84)* may be other strategies that can be borrowed in devising physical concentration measures.

Use of this effect for adsorptive bubble-based separation of microbial cells from bulk fluids is not a new phenomenon, and has in fact been capitalized upon for more than half a century *(87).* Originally adapted from the mining industry for separation and concentration of precious metals and minerals, flotation-based methods for recovery of microbial cells have been used advantageously by bioprocess engineers for separation of bacteria, yeasts, and algae from fluid bioreactors *(69, 85, 87).* Similar applications for foods may therefore be informed by the extensive knowledge bases present in the allied fields of environmental microbiology and bioprocess engineering. Interestingly, one early study using bubble-mediated processes for enrichment of *Serratia marcescens* from liquid suspensions showed that pigmented variants of this organism were concentrated to a higher degree than were nonpigmented strains *(16).* This type of effect, attributed to strain-to-strain, pigment-mediated differences in cell surface hydrophobicity, may indicate the potential for selective concentration of pigmented bacteria of importance to food microbiologists, such as some strains of *Cronobacter* (formerly *Enterobac-*

ter) *sakazakii.* Taking fresh perspectives on old problems by borrowing concepts from fields outside of food microbiology may be a powerful means for effecting change and advancing the state of the art for pre-analytical food sample preparation.

Another set of principles that may facilitate the concentration and separation of pathogens from foods capitalizes on the active participation of living microbes. Because these approaches depend on a contribution from the microbes themselves (motility and taxis or chemical change, for example), they might be termed ''collaborative'' in nature—with the microbe signaling its presence and facilitating its own recovery from the food. A good example of a collaborative approach is optimized Penn State University broth *(37).* Food samples inoculated into this semisolid medium pass first through an anaerobic recovery stage at the bottom of the tube, and then migrate to the surface of the medium where they effect a color change (formation of a black zone due to esculin hydrolysis). The selective agents in Penn State University broth suppress the growth of competitive microbiota, such as *Bacillus* spp., yet still allow recovery of injured listeriae. Migration of *Listeria* to the surface of the medium effectively separates motile (e.g. viable) cells from the food matrix and its associated inhibitors, and allows the cells to signal their presence through a color change; it also concentrates them where they can be easily accessed for additional testing if so desired *(37).*

Another motility-based collaborative approach for the separation and concentration of microorganisms from complex (nonfood) samples includes ''baiting,'' in which motile target cells are enriched at a solid surface, usually immersed in liquid. Solid surfaces can be the sole source of carbon added to the system (as with paraffin, described below), or a substrate to which the microorganisms are naturally attracted and preferentially colonize. Examples include paraffin baiting, which has been used to isolate acid-fast bacteria from soils or *Nocardia* spp. and *Pseudomonas aeruginosa* from clinical samples *(22, 47, 48),* and the use of pollen or hair, which can serve as baits for the isolation of motile zoospores of *Actinoplanes* spp. from soil *(60).* More recently, a bacterial ''trap'' has been described for the improved isolation of actinobacteria from soil or sediment *(25).* The device is a diffusion chamber containing sterile agar, bounded on either end by semipermeable membranes. The bottom membrane, which is in contact with the soil sample, has a 0.2-μm pore size that permits the free diffusion of nutrients and allows entry of filamentous bacteria and their subsequent growth, while excluding fungi. The trap was found to boost cultivation rates from marine sediments from 0.05% on petri dishes to 40%, thereby permitting the isolation of rare and previously ''uncultivable'' bacteria *(25).* The timeframes for these approaches, as described, typically range from 2 days to several weeks and rely not only on attraction of existing cells to the bait, but also on concomitant enrichment via growth. A method much more rapid would need to be devised if a similar approach were to be adapted for the ''collaborative'' isolation of pathogens from foods. However, perhaps our expanding knowledge of bacterial chemical communication will enable the syntheses of organism-specific signaling molecules or their analogs, which then may be used as chemoattractants to lure these target organisms into capillaries as a means of isolating them from food slurries—perhaps for direct processing and analysis via capillary-based, so-called lab-on-a-chip–type detection systems.

''Cutting out the middleman'': direct extraction of microbial analytes from the food matrix. Although our primary focus so far has been on the need to separate and concentrate intact (and ideally *living*) microbes from complex food matrices, there are some instances in which a route more direct from matrix to measurement may be possible. For tests such as PCR and some types of immunoassay, which detect ''disembodied'' cellular components, we can think of the cell simply as a carrier for freighting and delivery of the target analyte to a point of analysis. Because of their relatively large sizes, microbial cells are inherently easier to manipulate via physical means such as centrifugation and filtration than are individual macromolecules. Once delivered to a suitable collection point (i.e., the surface of a filter, the bottom of a microcentrifuge tube), cells may be lysed to release the target analyte for detection. For highly labile analytes such as some forms of RNA, the ''packaging'' provided by the microbial cell may help ensure intact delivery to the point of analysis.

In some situations, it may be possible to cut out the middleman (the need to deal with intact microbial cells), so to speak, by extracting diagnostic microbial analytes directly from the food matrix. The direct extraction of mycotoxins from foods prior to their detection sets a precedent for this type of approach, where physicochemical methods such as supercritical fluid extraction can be used to remove microbial analytes such as mycotoxins from bulk food matrices like flour *(98)*; however, mycotoxins are relatively hardy molecules secreted by toxigenic fungi into their local environments. It may be possible to use similar bulk extraction processes for other analytes more labile that are compartmented (and protected) within intact microbial cells distributed throughout a food, with the cells, their contents, and the surrounding food matrix treated as a single bulk sample from which target macromolecular analytes are extracted for detection.

Compared with standard microbiological means for detecting cell growth (i.e., appearance of a colony on a plate or an increase in turbidity of a liquid culture), the tools of analytical chemistry and molecular biology are orders of magnitude more sensitive, allowing detection of just a few molecules. With this type of approach, the problem can be (literally) boiled down to an exercise in detection of microbial macromolecules instead of microbial cells, and preparative chemical methods such as solvent extraction or high-performance liquid chromatography (HPLC) can also be leveraged to sift through the chaff for the molecule(s) of interest. A recent example of this type of approach is the use of a guanidinium thiocyanate–phenol–chloroform extraction and physical disruption with zirconium beads for the direct isolation of *Lactobacillus lactis* rRNA and mRNA from cheese *(49).* These authors found that this in-

tense physicochemical treatment was capable of simultaneously disrupting the cheese matrix, lysing cells of this starter culture, and inhibiting endogenous RNases that may have otherwise degraded target RNA in the sample.

Thinking even further outside the box, Lang et al. *(41)* used supercritical fluid extraction and HPLC analysis to detect microbial reduction-oxidation components (e.g., riboflavin, flavin adenine dinucleotide, or hemin) from bacteria associated with sand and soil, with an ultimate intent of developing tools capable of detecting extraterrestrial microbial life. A similar approach for detection of other microbial biomarkers (neutral lipids, phospholipids, dipicolinic acid, for example) from environmental and clinical samples suggests that, in some cases, food microbiologists may also cut out the middleman through direct extraction of microbial analytes from food matrices *(91).* While this approach may be desirable in some situations, a critical limitation is that subsequent cultural analysis is not possible, as it would be with methods capable of extracting living microbial cells from foods.

CURRENT REALITIES: IMPROVING ENRICHMENT-BASED METHODS

Shortening lag time and cultivating the uncultivable. Recognizing the limitations of cultural enrichment, and ultimately aspiring to methods that forgo this step, we should also recognize that enrichment, in some form, will realistically be a feature of the microbiological landscape for some time to come, and that it still has a valid role to play in methods for microbial detection. This stated, we might also aspire to improve it. A key drawback to enrichment processes is that when microbial cells are transferred to a new environment, they exhibit a lag phase—a period during which cell division is arrested. Typically explained simply as an ''adjustment'' period in which cells are adapting to new conditions, recent evidence suggests that bacteria experience true stresses during transfer from one medium to another. Cuny et al. *(19)* used strains harboring appropriate *lacZ*-reporter fusions and proteomic analyses to demonstrate that transfer of exponential-phase *E. coli* to agar plates immediately induced global stress regulons including those for heat shock and oxidative stress. The lag phase is of considerable practical importance to food microbiologists, as it may add several hours to overall time to detection for assays dependent on an enrichment step *(19).* New knowledge of what microbial cells are experiencing at a molecular level during a transfer from one medium to another, or perhaps more significantly, from a stressful food environment to laboratory growth media, may suggest approaches for shortening or even someday bypassing any significant lag phase.

Observations of microbial responses to microgravity and advances in our understanding of bacterial chemical communications may also pay dividends in our efforts to shorten or eliminate the lag phase. Remarkably, experiments conducted in space and in simulated microgravity conditions on Earth (low-shear modeled microgravity [LSMMG]) show significant effects on microbial growth kinetics, including shortening of the lag phase and generation times, extension of exponential growth, and higher final growth yields as compared with controls grown under normal gravitational conditions *(56).* For example, for *E. coli* grown in liquid media aboard the space shuttle, lag times were up to *4 to 8 h earlier* than for matched ground controls *(33, 36),* and the lag phase for *Saccharomyces cerevisiae* grown under LSMMG conditions was shortened by 90 min as compared with cultures grown under ''regular'' conditions *(67).* Similarly, generation times for *Salmonella* Typhimurium grown under LSMMG conditions were also noted to be 25 to 30 min shorter than for controls grown under normal conditions of gravity *(56).* The mechanisms underlying these observations have not been elucidated, but could stem from altered mass transfer equilibria governing nutrient uptake and metabolite removal from the cell's immediate environment, or from effects of low gravity on mechanosensitive processes that microbes may use to sense and respond to their environments *(36, 56).* These observations suggest that culture under microgravity or LSMMG conditions may have significant implications for modulation of bacterial growth kinetics, with potential applications to food microbiology. Further studies might focus on the effects of microgravity or LSMMG on growth kinetics of cells grown in the selective media used for enrichment of foodborne pathogens and the impact of these conditions on cellular production of target macromolecules (surface antigens, mRNA, rRNA, etc.) used for rapid detection of these pathogens.

Another fundamental limitation of enrichment stems from the fact that some organisms are uncultivable on artificial media, either because these media do not meet some unknown nutritional requirement of the organism, or because some aspect of the cell's physiological state prevents it from growing (i.e., the cell is in a viable but not culturable, or VBNC state). Apart from the use of diffusion chambers or ''bacterial traps,'' which allow growth of otherwise-uncultivable organisms under the ambient nutritional conditions of their natural environments *(25, 34),* other approaches may also be leveraged to initiate or promote the growth of uncultivable microorganisms, including the use of spent media containing unspecified, autostimulatory compounds *(90, 95),* coincubation with protective ''helper'' cultures *(50),* or the addition of defined signaling molecules, such as short peptides *(55).* In the case of spent or ''conditioned'' media (sterilized supernatants from cultures of either stationary-phase *E. coli* or exponential-phase *Listeria innocua*), these were found to shorten lag times for *E. coli* and *L. innocua* by up to ~50% *(90, 95).* Although the exact nature of the autostimulatory compound(s) present in these supernatants is not known, the effect is nonetheless remarkable. If such an approach could be used to reliably promote the growth of wild-type food pathogen isolates, the undefined and ''raw'' nature of this technique could make it especially valuable, as it should be relatively simple and low cost to implement (versus addition of a potentially costly, defined synthetic agent to media). To avoid false positives from inadvertent inoculation of otherwise-negative enrichment samples, great care would be required to ensure sterility of the added supernatant.

The recent use of helper bacteria to facilitate growth of *Prochlorococcus* spp. in laboratory media provides another example of how cultural conditions might be modified to enhance an enrichment outcome for an organism that does not respond to traditional conditions. Although no immediate corollary for this example is apparent for food-based applications, it was hypothesized that these helper cultures may be instrumental in reducing oxidative stresses experienced by *Prochlorococcus* spp., stresses already known to limit the growth of sublethally injured foodborne pathogens *(79).* Such helper cultures might also actively produce signaling molecules capable of initiating or stimulating the growth of otherwise-uncultivable bacteria. A case in point is the recent discovery of a biologically active eight–amino acid peptide capable of stimulating the growth of a marine bacterium isolate *(55).* It is not known if this peptide is identical to, or simply similar to, a factor expressed by a cultivable helper organism observed to promote the growth of this isolate, but its activity is clear. Interestingly, the same uncultivable marine isolate also responded to unknown factors expressed by *Salmonella* Typhimurium, highlighting the potential for wider cross-species chemical communications that may have direct applications in food microbiology.

In summary, there are a number of potential tricks that food microbiologists might be able to borrow from environmental microbiology. These may allow us to shorten lag time, cultivate otherwise-uncultivable organisms, and manipulate the population composition of nonselective enrichments. Together, these potential strategies highlight the notion that while cultural enrichment is not quite perfect, it is also not quite dead, and therefore may still remain valuable for some time to come.

Alternative dilutions. It is important to recognize that standard methods developed by governmental regulatory agencies and now widely used by food microbiologists worldwide were generated in response to prevailing conditions of the day. Because nothing in science is static, these methods may contain historical elements or motivations, and should therefore not be considered as exempt from periodic reevaluation. One canonical element in food microbiology that may bear reexamination is the decimal dilution.

Dilution serves a number of functions: it enables us to count (via extrapolation from the 25 to 250 countable CFU on a plate) the number of CFU present in the original, undiluted sample; it effectively removes food particles, intense coloration, or opacity that may interfere with accurate counting of colonies on a plate; and it facilitates removal of inhibitory substances such as antimicrobials or metabolic end products that may interfere with cell growth. Dilution also removes biochemical inhibitors that may interfere with molecular methods such as PCR. Decimal dilutions not only effectively serve these functions, but they are also intuitively calculated, and are therefore convenient and simple. The standard analytical unit is 25 g of food; however, when pathogens may be present at very low levels and/or may be heterogeneously distributed within a bulk food sample, the use of a larger analytical unit can increase chances for detection *(62, 63, 97).* If the same dilution ratio is used, though, the amount of media required may quickly become burdensome and expensive, especially if a large number of increased-volume samples must be examined *(63, 97).* Thus, the cost of microbiological media may represent a significant economic barrier to the use of nonstandard procedures, such as examination of larger analytical units.

To address these concerns, several laboratories have recently sought to compare the performance of alternative dilutions and/or less expensive nonselective media against that of more commonly used dilutions (i.e., 1:10) and more complex, selective media for recovery of various pathogens. For example, Guerini et al. *(26)* evaluated 12 different media for their abilities to support the growth of *E. coli* O157:H7. Both selective and nonselective media were examined at 37 or 42°C, under static or shaking conditions, and doubling times for each condition were calculated. The eight top-performing media from this initial round of testing were scrutinized further for practical factors, including cost and ease of use *(26).* The best-performing, most practical medium was found to be Trypticase soy broth (TSB), with static incubation at 42°C. Initial data from this study also indicated that a 1:3 ratio of sample to medium was equivalent to a 1:10 ratio in a test-and-hold procedure for *E. coli* O157:H7 on beef samples *(26).* In a subsequent study by this same group, Bosilevac and Koohmaraie *(13)* investigated the use of reduced volumes of TSB for enrichment of *E. coli* O157:H7 in raw beef prior to detection via PCR, including two commercial tests. Volumes tested were 3, 1, 0.5, and 0× TSB, where the 0× treatment represented incubation of the meat without added medium, which was followed by a postincubation homogenization in 1 volume of TSB. These authors found that presence or absence detection of *E. coli* O157:H7 in ground beef via immunomagnetic separation culture or PCR was not affected by the volume of TSB used for enrichment, and that optimal results for all test and sample conditions examined were obtained by using 1 volume of TSB per unit of sample. In another study reassessing both media requirements and enrichment ratios, Oyarzabal et al. *(63)* investigated buffered peptone water supplemented with blood and antibiotics as a potential replacement for Bolton broth for enumeration of thermotolerant *Campylobacter* spp., and also compared a 1:4 enrichment ratio with the standard ratio of 1:9. These authors found that modified buffered peptone water at an enrichment ratio of 1:4 was statistically similar to Bolton broth or buffered peptone water used in the traditional ratio of 1:9 *(63).*

Together, these studies suggest the utility (and the possibility) of using nonstandard or alternative dilutions and/or nonselective media in enrichments for various pathogens. Apart from the economic advantages associated with reduced media consumption, the ability to use lower levels of media and/or nonselective media may have additional benefits, such as physical concentration of cells from large analytical units into relatively small amounts of media and assay simplicity. Potential drawbacks of this type of approach may be the effects on the growth of background

microbiota at different sample to medium ratios, especially for nonselective enrichments *(13),* and a lack of dilution for foods formulated with preservatives or other growth inhibitors *(97).* Additionally, the higher fat or biochemical inhibitor content expected in less dilute media could affect recovery rates via immunomagnetic separation or amplification via PCR.

PUSHING FORWARD NEXT-GENERATION SYSTEMS: PRACTICAL CONSIDERATIONS

Keep it simple: sampler. Complex is not always better. Although some sampling, separation, or concentration approaches may involve specialized instrumentation (e.g., wet vacuum–based surface sampling, recirculating immunocapture, continuous flow centrifugation), some samples may be amenable to the use of approaches much simpler and less capital intensive. Examples include the use of Scotch tape (or similar adhesive tapes) for sampling of produce, meats, or food contact surfaces *(9, 24)*; Kimwipe absorbent tissues for recovery of *Listeria monocytogenes* from stainless steel surfaces *(86)*; and simple filtration or centrifugation steps for PCR-based detection of *Salmonella* or *E. coli* O157:H7 from chicken rinsate, alfalfa sprouts or mung bean, and sprout irrigation waters *(32, 93).* Advantages of very simple methods for sampling or sample preparation include their relative ease to learn, apply, and troubleshoot, the wide availability of the raw materials or equipment needed to conduct the assay, speed (as a result of fewer and/or more rapidly accomplished steps), acceptable or improved efficacy or reproducibility vis-à-vis existing methods, and reduced expense on both per-assay and capital investment bases.

Magnetic capture hybridization. Elsewhere in this article, we have promoted the idea of reframing our thinking about whole microorganisms to treat them as simple biochemical analytes. Because turnabout is fair play, we may also leverage methods that have been primarily applied to whole cells for the separation and concentration of biochemical analytes such as DNA from complex samples. An example is magnetic capture hybridization (MCH), a conceptual cousin to immunomagnetic separation. In the MCH approach, magnetic particles are decorated with nucleic acid probes designed for hybridization-based capture of specific nucleic acid sequences.

MCH is beneficial on at least two levels: it provides a physical approach for purification of nucleic acids away from inhibitory substances found in the food matrix, but it also enriches for specific nucleic acid sequences, allowing these to be selectively purified away from nontarget nucleic acids that could arise from either nontarget bacteria or from the food matrix itself. Downstream detection of MCH-captured nucleic acids can then be performed via either PCR *(1)* or reverse transcription PCR *(31),* and has been applied to the detection of *L. monocytogenes* in milk *(1)* or *Salmonella* in artificially contaminated soil or chicken manure *(31).*

Alternative binders. Molecular-recognition events play critical roles throughout the biological world, mediating processes as diverse as DNA replication, genetic regulation of biochemical pathways, selective uptake of nutrients, directed transport of proteins within a cell, chemotaxis, cell-cell communication, and attachment of bacteria to host cells or of phage to bacteria. Antibody binding and nucleic acid hybridization exemplify how the power of these naturally evolved molecular-recognition mechanisms can be harnessed for use in diagnostics, allowing detection of specific cellular components. Other types of naturally occurring binder-ligand interactions can also be exploited for detection of target biomolecules. At the level of the whole cell, these might also be used as a means for separation and concentration of target organisms from physically and microbiologically complex food environments.

Theoretically, any type of interaction that allows capture and/or concentration of biomolecules or cells can be used diagnostically. These include interactions based on simple, charge-based affinities, as well as more specific mechanisms, such as lock-and-key docking. For some simple capture applications, high specificity is not prerequisite—bioaffinity reagents capable of nonspecifically adsorbing microbial cells could be used for first-level capture of a variety of microbial cells or structures present in a sample, similar to the present use of metal hydroxides or silica particles for this purpose *(80, 96).* Natural molecules that have attracted interest as alternative binders include antibiotics, proteins such as lectins and pig gastric mucin *(83),* or peptide binders, including antimicrobial peptides *(2, 54).* Synthetic or engineered binder molecules include DNA or RNA aptamers *(54, 65),* recombinant antibodies or antibody fragments *(21),* combinatorial binders based on randomizable protein scaffolds *(38, 61, 75),* artificial antibodies, or ''plastibodies'' based on molecularly imprinted polymers *(54),* and synthetic glycopolymers *(66).*

The effective use of an alternative binder involving magnetic beads functionalized with porcine gastric mucin for pre-PCR binding and concentration of human noroviruses has been demonstrated *(17, 83).* Histo-blood group antigens are carbohydrate eptitopes present on human gut mucosal cell surfaces that serve as receptors for noroviruses *(28).* Tian et al. *(83)* found that porcine gastric mucin contains a mixture of histo-blood group antigens able to bind human noroviruses, and took advantage of this result to develop a porcine gastric mucin–mediated magnetic capture method for concentrating noroviruses belonging to genogroups I and II. Using this approach, these authors were able to concentrate and detect noroviruses in spiked feces, with a 2-log improvement in sensitivity over their existing RNA extraction procedure *(83).* Cannon and Vinje *(17)* demonstrated the utility of histo-blood group antigen–coated magnetic beads to recover low levels of noroviruses from environmental waters. Polyclonal antibodies have been used similarly in the magnetic concentration of noroviruses; however, human norovirus antibodies are typically strain specific, which may significantly affect the development of broadly reactive, sensitive reagents. The widespread use of pigs as food animals provides a ready source of porcine gastric mucin, which could provide an

additional economic incentive for use of this protein for norovirus capture and concentration.

The dilemma of sublethally injured cells. Proof of concept work for most detection protocols is carried out by using laboratory cultures—pure cultures or well-defined mixtures of known isolates grown under optimal conditions. We expect these cells will be healthy and vigorous, producing abundant quantities of the target structures or molecules necessary for detection. Ideally, we would hope that cells extracted from foods would be in as good condition and as robust as those grown in media. For some combinations of pathogens, foods and growth conditions, we may be able to approach, if not meet, this ideal situation. More commonly, though, we can expect that our samples may contain cells exposed to stress conditions common to foods or food processing environments, including salt, extremes of temperature (hot or cold), limited water availability or desiccation, acidic conditions, nutrient limitation or starvation, natural or applied antimicrobials, buildup of toxic metabolites, or any combination of these. These conditions may induce physiological (and on occasion, genetic) responses that affect cellular characteristics such as cell shape, size, flagellar expression, surface charge or hydrophobicity, and macromolecular integrity or content. Unfortunately, these cellular characteristics may also be those we seek to take advantage of for separation, concentration, and/or detection of target cells from foods.

Presuming we are able to effectively separate stressed or dormant target cells from the food matrix and concentrate them into an analytically suitable volume, they still may contain degraded structures or low levels of target macromolecules, both of which could affect our abilities to detect them. Although an enrichment step may enable the repair, resuscitation, and robust growth needed to counter the negative effects of stresses from the food environment, enrichments are time-consuming and confound attempts at quantitative detection. In some cases, brief exposure of extracted cells to nutrients may facilitate sufficient production of target molecules to enhance detection within timeframes where substantial cell proliferation would not be expected to occur. An example is the brief postsampling exposure of *L. monocytogenes* to a dilute nutrient medium, which stimulates increased production of rRNA, facilitating detection of these cells by using a combination of *Listeria*-specific fluorescence in situ hybridization and flow cytometry *(15).* In this example, cells of *L. monocytogenes* that had been growing for 1 week on vacuum-packaged turkey hot dogs, under conditions of mild temperature abuse (8°C), were exposed to dilute (1:4) deMan Rogosa Sharpe broth and incubated at 30°C for 70 min. Cells treated in this fashion demonstrated increases in both light scatter and fluorescence, indicating the beginnings of cell elongation as might occur during the stationary phase, as well as enhanced production of the probe's rRNA target. This approach enabled full separation of the *L. monocytogenes* population from the particulate and nontarget microbial backgrounds when viewed by posthybridization flow cytometry. The term *nutrient amendment* may be used to describe this sort of preanalytical resuscitative step, differentiating this use of liquid growth media (or other nutrient source) from the multiplication-based term *enrichment.*

Recovery and detection of living cells. An inherent advantage of culture-based detection methods is the ability to confirm the viability of the isolate. Nucleic acid–based methods are limited in this regard because they cannot definitively differentiate living or dead pathogenic bacteria, as DNA is very stable and quite persistent in the environment. Recently, the DNA-intercalating agents ethidium monoazide and propidium monoazide have been used in conjunction with PCR for the selective detection of live cells of a variety of foodborne pathogens *(58, 59, 70, 88).* These compounds selectively penetrate the membranes of dead cells and form stable DNA monoadducts on photolysis, resulting in DNA that cannot be amplified. With further optimization, an approach such as this may help address the problem of detection of nonliving cells.

Although the current legal and regulatory climate has not yet addressed this issue, the time is coming when one or more pathogen-specific nucleic acid sequences will likely be evidence for contamination and may serve as a confirmation step. This may be particularly important for the nonculturable agents (such as the noroviruses). For the time being, PCR methods for pathogen screening are easily defensible if followed by culture-based confirmation.

Lysis and nucleic acid extraction. Because nucleic acid is an internal component of microbial pathogens, both lysis and subsequent nucleic acid purification must precede PCR. In fact, the reliability of nucleic acid–based detection approaches depends in large part on the purity of the target template and the number of target molecules. Therefore, DNA or RNA extraction methods have several purposes: (i) to make the nucleic acids available for amplification and detection, (ii) to provide for further sample concentration, and (iii) to remove residual matrix-associated inhibitory substances that might remain even after the initial pathogen concentration steps are completed. This is particularly apparent when dealing with low template level, which is in fact the issue with low levels of contamination. Over the course of the last decade, many commercial kits and even some automated systems have been produced to facilitate nucleic acid extraction, and some are designed specifically for use in complex sample matrices. While facilitating detection, further improvements to make these methods more user friendly with better extraction efficiencies are needed to further improve limits of detection that can be achieved by using PCR-based detection methods.

Validation and cost. The need for a new vision for preanalytical sample processing technology is clear, if we are to move foodborne pathogen detection methods forward so that they can be truly more rapid while also offering enumerative capabilities. As newer methods become available, they will need to be fully validated and standardized. In fact, some of this validation burden could potentially be alleviated by the introduction of sample purification and preparation methods to remove interfering compounds prior

to detection. Additionally, the introduction of sample purification might increase the range of sample types that could by analyzed by using a single-assay format.

Unfortunately, the introduction of sample preparation steps to the front end of detection will likely increase testing costs and complexity. Issues surrounding complexity can be alleviated by automation, again at a cost. The end user will need to recognize that improved time to result, and the greater value of enumerative data will not come without attention to preanalytical sample processing and its associated costs.

Instrumentation design: next-generation systems. A common trend in the design of new microbiological detection systems is the development of instruments that can be used at or near the location where the sample is obtained, as opposed to collecting the sample and transporting it to a laboratory for subsequent processing and detection. This trend is forcing instrument developers to build systems that are smaller, lighter, and more rugged than systems available today. Additionally, it is forcing instruments to become more user friendly for operation by less experienced or well-trained staff. This trend will drive future sample preparation approaches, since these methods will need to be automated and integrated along with next-generation detection instrumentation. These smaller and more user-friendly instrument profiles will also need to address sample concentration so that nucleic acid or immunological targets are presented to the detection module in smaller volumes.

Next-generation PCR systems under development simultaneously detect many different pathogens from a single sample. This is currently done either by multiplexing (performing many tests in the same sample volume) or by performing large numbers of independent, but parallel reactions. Regardless of the approach used, both methods rely on the ability to generically purify nucleic acids from a sample without regard to sequence. In other words, any DNA present in the sample will be purified regardless of the type of origin of that DNA. There are advantages to this approach: if only sequence-specific purification methods were available, then many different purification reactions would have to be performed to test a single sample. In this case, then, the sample preparation technology is actually facilitating the ability to detect multiple targets and hence multiple agents. The success of multiplexing and chip-based molecular approaches has led to a desire to use a similar approach for next-generation immunological detection systems whereby many different immunological targets could be detected simultaneously from a single sample; however, the lack of generic protein purification approaches is a significant impediment to these efforts.

Another trend prevalent in the development of rapid detection platforms is to use multiple different detection strategies to analyze a single sample. In the relatively near term, this would involve combining PCR- and immunological-based detection strategies into a single-instrument platform. The use of multiple detection strategies based on a highly parallel, redundant, orthogonal testing strategy could improve the confidence in and accuracy of rapid test results; however, this approach has significant implications for sample preparation, since the system would not only need to employ multiple detection methodologies, but would also be required to produce samples appropriate for two or more, perhaps very different, detection platforms. For this type of system, the best sample processing method would be to use a single, universal strategy capable of purifying all target molecules simultaneously, thereby eliminating the need to split the sample. However, there are no currently available methods capable of copurifying multiple targets, such as nucleic acids and proteins, or nucleic acids and cell membranes.

In the final analysis, the development of fieldable, integrated, and automated detection systems that are capable of multianalyte detection will drive the need for new and novel sample preparation strategies. For the most part, the sample preparation strategies needed for these next-generation systems do not exist today and a significant effort will be required for their development, automation, and integration.

SUMMARY

Over the past several decades, many improvements have been made in the microbiological analysis of foods, particularly with respect to detection platform. Enrichment culture, coupled with molecular recognition of microbial targets, currently provides the greatest sensitivity and specificity of detection. However, this workhorse of the microbiological laboratory is not applicable in important cases (e.g., viruses, parasites), is not readily amenable to quantitative needs, and perhaps most importantly, has not allowed us to circumvent the need for lengthy cultural enrichment. Together, these issues impede our progress toward truly rapid detection (time to result, <4 h) of pathogens in foods.

To make truly rapid detection a reality, we must devote major efforts and resources to developing strategies that will ultimately reduce our reliance on enrichment culture. Such strategies must involve a holistic consideration of the component parts (sampling, separation, concentration, detection) in an integrated analytical scheme. It is particularly critical to devote greater time and resources to developing practical, simple, and reliable separation and concentration technologies, with an eye on the microorganism as a physicochemical analyte. Perhaps the most difficult aspect of this task will be the methodical application of these technologies to the full spectrum of existing food commodities and potential contaminating microbes. Nevertheless, we need to begin somewhere, and a logical direction is by promoting interdisciplinary collaboration and directing adequate funding resources to solving this long-standing problem.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the International Association for Food Protection for their financial and organizational support of the Sample Prep Working Group, which provided the structure for discussions that resulted in this review.

REFERENCES

1. Amagliani, G., E. Omiccioli, A. del Campo, I. J. Bruce, G. Brandi, and M. Magnani. 2006. Development of a magnetic capture hybridization-PCR assay for *Listeria monocytogenes* direct detection in milk samples. *J. Appl. Microbiol.* 100:375–383.
2. Arcidiacono, S., P. Pivarnik, C. M. Mello, and A. Senecal. 2008. Cy5 labeled antimicrobial peptides for enhanced detection of *Escherichia coli* O157:H7. *Biosens. Bioelectron.* 23:1721–1727.
3. Armstrong, D. W., G. Schulte, J. M. Shneiderheinze, and D. J. Westenberg. 1999. Separating microbes in the manner of molecules. 1. Capillary electrokinetic approaches. *Anal. Chem.* 71:5465–5469.
4. Arora, K., S. Chand, and B. D. Malhotra. 2006. Recent developments in biomolecular electronics techniques for food pathogens. *Anal. Chim. Acta* 568:259–274.
5. Baylor, E. R., V. Peters, and M. B. Baylor. 1977. Water-to-air transfer of virus. *Science* 197:763–764.
6. Bennett, A. R., F. G. Davids, S. Vlahodimou, J. G. Banks, and R. P. Betts. 1997. The use of bacteriophage-based systems for the separation and concentration of *Salmonella*. *J. Appl. Microbiol.* 83:259–265.
7. Benoit, P. W., and D. W. Donahue. 2003. Methods for rapid separation and concentration of bacteria in food that bypass time-consuming cultural enrichment. *J. Food Prot.* 66:1935–1948.
8. Berry, E. D., and G. R. Siragusa. 1997. Hydroxyapatite adherence as a means to concentrate bacteria. *Appl. Environ. Microbiol.* 63:4069–4074.
9. Bisha, B., and B. F. Brehm-Stecher. 2009. Simple adhesive-tape-based sampling of tomato surfaces combined with rapid fluorescence in situ hybridization for *Salmonella* detection. *Appl. Environ. Microbiol.* 75:1450–1455.
10. Blanchard, D. C. 1989. The ejection of drops from the sea and their enrichment with bacteria and other materials: a review. *Estuaries* 3:127–137.
11. Blanchard, D. C., and L. D. Syzdek. 1982. Water-to-air transfer and enrichment of bacteria in drops from bursting bubbles. *Appl. Environ. Microbiol.* 43:1001–1005.
12. Blanchard, D. C., L. D. Syzdek, and M. E. Weber. 1981. Bubble scavenging of bacteria in freshwater quickly produces bacterial enrichment in airborne jet drops. *Limnol. Oceanogr.* 26:961–964.
13. Bosilevac, J. M., and M. Koohmaraie. 2008. Effects of using reduced volumes of nonselective enrichment medium in methods for the detection of *Escherichia coli* O157:H7 from raw beef. *J. Food Prot.* 71:1768–1773.
14. Brehm-Stecher, B. F., and E. A. Johnson. 2004. Single-cell microbiology: tools, technology and applications. *Microbiol. Mol. Biol. Rev.* 68:538–559.
15. Brehm-Stecher, B. F., and E. A. Johnson. 2007. Rapid methods for detection of *Listeria,* p. 268. *In* E. Marth and E. Ryser (ed.), *Listeria,* listeriosis and food safety, 3rd ed. Marcel Dekker, New York.
16. Burger, S. R., and J. W. Bennett. 1985. Droplet enrichment factors of pigmented and non-pigmented *Serratia marcescens*: possible selective function for prodigiosin. *Appl. Environ. Microbiol.* 50:487–490.
17. Cannon, J. L., and J. Vinje. 2008. Histo-blood group antigen assay for detecting noroviruses in water. *Appl. Environ. Microbiol.* 74:6818–6819.
18. Carlucci, A. F., and P. M. Williams. 1965. Concentration of bacteria from sea water by bubble scavenging. *J. Cons. Int. Explor. Mer* 30:28–33.
19. Cuny, C., M. Lesbats, and S. Dukan. 2007. Induction of a global stress response during the first step of *Escherichia coli* plate growth. *Appl. Environ. Microbiol.* 73:885–889.
20. De Martinis, E. C. P., R. E. Duvall, and A. D. Hitchins. 2007. Real-time PCR detection of 16S rRNA genes speeds most-probable-number enumeration of foodborne *Listeria monocytogenes*. *J. Food Prot.* 70:1650–1655.
21. Fernández, L. A. 2004. Prokaryotic expression of antibodies and affibodies. *Curr. Opin. Biotechnol.* 15:364–373.
22. Frey, C. A. 1930. A method for detecting acid-fast bacteria in the soil. *Science* 71:366.
23. Fukushima, H., K. Katsube, Y. Hata, R. Kishi, and S. Fujiwara. 2007. Rapid separation and concentration of food-borne pathogens in food samples prior to quantification by viable-cell counting and real-time PCR. *Appl. Environ. Microbiol.* 73:92–100.
24. Fung, D. Y. C., L. K. Thompson, B. A. Crozier-Dodson, and C. L. Kastner. 2000. Hands-free, ''pop-up,'' adhesive tape method for microbial sampling of meat surfaces. *J. Rapid Methods Autom. Microbiol.* 8:209–217.
25. Gavrish, E., A. Bollmann, S. Epstein, and K. Lewis. 2008. A trap for in situ cultivation of filamentous actinobacteria. *J. Microbiol. Methods* 72:257–262.
26. Guerini, M. N., T. M. Arthur, S. D. Schackelford, and M. Koohmaraie. 2006. Evaluation of *Escherichia coli* O157:H7 growth media for use in test-and-hold procedures for ground beef processing. *J. Food Prot.* 69:1007–1011.
27. Gunasekera, T. S., P. V. Attfield, and D. A. Veal. 2000. A flow cytometry method for rapid detection and enumeration of total bacteria in milk. *Appl. Environ. Microbiol.* 66:1228–1232.
28. Harrington, P. R., J. Vinjé, C. L. Moe, and R. S. Baric. 2004. Norovirus capture with histo-blood group antigens reveals novel virus-ligand interactions. *J. Virol.* 78:3035–3045.
29. International Commission on Microbiological Specifications for Foods. 1986. Microorganisms in foods 2. Sampling for microbiological analysis: principles and specific applications, 2nd ed. University of Toronto Press, Toronto.
30. International Commission on Microbiological Specifications for Foods. 2002. Microorganisms in foods 7. Microbiological testing in food safety management. Kluwer Academic/Plenum Publishers, New York.
31. Jacobsen, C. S., and W. E. Holben. 2007. Quantification of mRNA in *Salmonella* sp. seeded soil and chicken manure using magnetic capture hybridization RT-PCR. *J. Microbiol. Methods* 69:315–321.
32. Johnston, L. M., D. Elhanafi, M. Drake, and L.-A. Jaykus. 2005. A simple method for the direct detection of *Salmonella* and *Escherichia coli* O157:H7 from raw alfalfa sprouts and irrigation water using PCR. *J. Food Prot.* 68:2256–2263.
33. Kacena, M., and P. Todd. 1997. Growth characteristics of *E. coli* and *B. subtilis* cultured on an agar substrate in microgravity. *Microgravity Sci. Technol.* 10:58–62.
34. Kaeberlein, T., K. Lewis, and S. S. Epstein. 2002. Isolating ''uncultivable'' microorganisms in pure culture in a simulated natural environment. *Science* 296:1127–1129.
35. Kearns, E. A., S. Magaña, and D. V. Lim. 2008. Automated concentration and recovery of micro-organisms from drinking water using dead-end ultrafiltration. *J. Appl. Microbiol.* 105:432–442.
36. Klaus, D., S. Simske, P. Todd, and L. Stodieck. 1997. Investigation of space flight effects on *Escherichia coli* and a proposed model of the underlying physical mechanisms. *Microbiology* 143:449–455.
37. Knabel, S. J. 2002. Optimized, one-step recovery-enrichment for enhanced detection of *Listeria monocytogenes* in pasteurized milk and hot dogs. *J. AOAC Int.* 85:501–504.
38. Kolmar, H. 2008. Alternative binding proteins: biological activity and therapeutic potential of cystine-knot miniproteins. *FEBS J.* 275:2684–2690.
39. Kostal, V., and E. A. Arriaga. 2008. Recent advances in the analysis of biological particles by capillary electrophoresis. *Electrophoresis* 29:2578–2586.
40. Lambertini, E., S. K. Spencer, P. D. Bertz, F. J. Loge, B. A. Kieke, and M. A. Borchardt. 2008. Concentration of enteroviruses, adenoviruses, and noroviruses from drinking water by use of glass wool filters. *Appl. Environ. Microbiol.* 74:2990–2996.
41. Lang, Q., I. F. Cheng, C. M. Wai, A. Paszczynski, R. L. Crawford, B. Barnes, T. J. Anderson, R. Wells, G. Corti, L. Allenbach, D. P. Erwin, T. Assefi, and M. Mojarradi. 2002. Supercritical fluid extraction and high-performance liquid chromatography-diode array-electrochemical detection of signature redox compounds from sand and soil samples. *Anal. Biochem.* 301:225–234.
42. Lantz, A. W., B. F. Brehm-Stecher, and D. W. Armstrong. 2008. Combined capillary electrophoresis and DNA-FISH for rapid mo-

lecular identification of *Salmonella* Typhimurium in mixed culture. *Electrophoresis* 29:2477–2484.
43. Lazcka, O., F. J. Del Camp and F. X. Munoz. 2007. Pathogen detection: a perspective of traditional methods and biosensors. *Biosens. Bioelectron.* 22:1205–1217.
44. Lindquist, H. D. A., S. Harris, S. Lucas, M. Hartzel, D. Riner, P. Rochele, and R. DeLeon. 2007. Using ultrafiltration to concentrate and detect *Bacillus anthracis, Bacillus atrophaeus* subspecies *globigii,* and *Cryptosporidium parvum* in 100-liter water samples. *J. Microbiol. Methods* 70:484–492.
45. Lucore, L. A., M. A. Cullison, and L.-A. Jaykus. 2000. Immobilization with metal hydroxides as a means to concentrate food-borne bacteria for detection by cultural and molecular methods. *Appl. Environ. Microbiol.* 66:1769–1776.
46. Marks, T., and R. Sharp. 2000. Bacteriophages and biotechnology: a review. *J. Chem. Technol. Biotechnol.* 75:6–17.
47. Massengale, A. R. D., R. A. Ollar, S. J. Giordano, M. S. Felder, and S. C. Aronoff. 1999. Use of the paraffin wax baiting system for identification of *Pseudomonas aeruginosa* clinical isolates. *Diagn. Microbiol. Infect. Dis.* 35:177–183.
48. Mishra, S. K., and H. S. Randhawa. 1969. Application of paraffin bait technique to the isolation of *Nocardia asteroides* from clinical specimens. *Appl. Microbiol.* 18:686–687.
49. Monnet, C., V. Ulvé, A.-S. Sarthou, and F. Irlinger. 2008. Extraction of RNA from cheese without prior separation of microbial cells. *Appl. Environ. Microbiol.* 74:5724–5730.
50. Morris, J. J., R. Kirkegaard, M. J. Szul, Z. I. Johnson, and E. R. Zinser. 2008. Facilitation of robust growth of *Prochlorococcus* colonies and dilute liquid cultures by ''helper'' heterotrophic bacteria. *Appl. Environ. Microbiol.* 74:4530–4534.
51. Mottram, T., A. Rudnitskaya, A. Legin, J. L. Fitzpatrick, and P. D. Eckersall. 2007. Evaluation of a novel chemical sensor system to detect clinical mastitis in bovine milk. *Biosens. Bioelectron.* 22: 2689–2693.
52. Nakamura-Tsuruta, S., Y. Kishimoto, T. Nishimura, and Y. Suda. 2008. One-step purification of lectins from banana pulp using sugar-immobilized gold nano-particles. *J. Biochem.* 143:833–839.
53. Neeley, E. T., T. G. Phister, and D. A. Mills. 2005. Differential real-time PCR assay for enumeration of lactic acid bacteria in wine. *Appl. Environ. Microbiol.* 71:8954–8957.
54. Ngundi, M. M., N. V. Kulagina, G. P. Anderson, and C. R. Taitt. 2006. Nonantibody-based recognition: alternative molecules for detection of pathogens. *Expert Rev. Proteomics* 3:511–524.
55. Nichols, D., K. Lewis, J. Orjala, S. Mo, R. Ortenberg, P. O'Connor, C. Zhao, P. Vouros, T. Kaeberlein, and S. S. Epstein. 2008. Short peptide induces an ''uncultivable'' microorganism to grow in vitro. *Appl. Environ. Microbiol.* 74:4889–4897.
56. Nickerson, C. A., C. M. Ott, J. W. Wilson, R. Ramamurthy, and D. L. Pierson. 2004. Microbial responses to microgravity and other low-shear environments. *Microbiol. Mol. Biol. Rev.* 68:345–361.
57. Nitsche, A., A. Kurth, A. Dunkhorst, O. Pänke, H. Sielaff, W. Junge, D. Muth, F. Scheller, W. Stöcklein, C. Dahmen, G. Pauli, and A. Kage. 2007. One-step selection of Vaccinia virus–binding DNA aptamers by MonoLEX. *BMC Biotechnol.* 7:48.
58. Nocker, A., C. Y. Cheung, and A. K. Camper. 2006. Comparison of propidium monoazide with ethidium monoazide for differentiation of live versus dead bacteria by selective removal of DNA from dead cells. *J. Microbiol. Methods* 67:310–320.
59. Nogva, H. K., S. M. Dromtorp, H. Nissen, and K. Rudi. 2003. Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5′-nuclease PCR. *Biotechniques* 34:804.
60. Nonomura, H., and S. Takagi. 1977. Distribution of actinoplanetes in soils of Japan. *J. Ferment. Technol.* 55:423–428.
61. Nygren, P.-A. 2008. Alternative binding proteins: affibody binding proteins developed from a small three-helix bundle scaffold. *FEBS J.* 275:2668–2676.
62. Oscar, T. P. 2004. Predictive simulation model for enumeration of *Salmonella* on chicken as a function of polymerase chain reaction detection time score and sample size. *J. Food Prot.* 67:1201–1208.
63. Oyarzabal, O. A., S. Backert, M. Nagaraj, R. S. Miller, S. K. Hussain, and E. A. Oyarzabal. 2007. Efficacy of supplemented buffered peptone water for the isolation of *Campylobacter jejuni* and *C. coli* from broiler retail products. *J. Microbiol. Methods* 69:129–136.
64. Palchetti, I., and M. Mascini. 2008. Electroanalytical biosensors and their potential for food pathogen and toxin detection. *Anal. Bioanal. Chem.* 391:455–471.
65. Pan, Q., X.-L. Zhang, H.-Y. Wu, P.-W. He, F. Wang, M.-S. Zhang, J.-M. Hu, B. Xia, and J. Wu. 2005. Aptamers that preferentially bind type IVB pili and inhibit human monocytic-cell invasion by *Salmonella enterica* serovar Typhi. *Antimicrob. Agents Chemother.* 49: 4052–4060.
66. Pasparakis, G., and C. Alexander. 2007. Synthetic polymers for capture and detection of microorganisms. *Analyst* 132:1075–1082.
67. Purevdorj-Gage, B., K. B. Sheehan, and L. E. Hyman. 2006. Effects of low-shear modeled microgravity on cell function, gene expression, and phenotype in *Saccharomyces cerevisiae. Appl. Environ. Microbiol.* 72:4569–4575.
68. Rossmanith, P., B. Süß, M. Wagner, and I. Hein. 2007. Development of matrix lysis for concentration of gram positive bacteria from food and blood. *J. Microbiol. Methods* 69:504–511.
69. Rubin, A. J., E. A. Cassel, O. Henderson, J. D. Johnson, and J. C. Lamb III. 1966. Microfloatation: new low gas-flow rate foam separation technique for bacteria and algae. *Biotechnol. Bioeng.* 8:135–151.
70. Rudi, K., K. Naterstad, S. M. Drømtorp, and H. Holo. 2005. Detection of viable and dead *Listeria monocytogenes* on gouda-like cheeses by real-time PCR. *Lett. Appl. Microbiol.* 40:301–306.
71. Russ, C. 1909. The electrical reactions of certain bacteria, and an application in the detection of tubercle bacilli in urine by means of an electric current. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 81:314–322.
72. Schindler, B. D., and L. A. Shelef. 2006. Immobilization and detection of *Listeria monocytogenes. Appl. Environ. Microbiol.* 72:4426–4428.
73. Seo, K.-H., P. S. Holt, H. D. Stone, and R. K. Gast. 2003. Simple and rapid methods for detecting *Salmonella enteritidis* in raw eggs. *Int. J. Food Microbiol.* 87:139–144.
74. Sharpe, A. N. 2003. Separation and concentration of samples, p. 52–68. *In* T. A. McMeekin (ed.), Detecting pathogens in food, 1st ed. CRC Press, Boca Raton, FL.
75. Skerra, A. 2008. Alternative binding proteins: anticalins—harnessing the structural plasticity of the lipocalin ligand pocket to engineer novel binding activities. *FEBS J.* 275:2677–2683.
76. Skjerve, E., L. M. Rorvik, and O. Olsvik. 1990. Detection of *Listeria monocytogenes* in foods by immunomagnetic separation. *Appl. Environ. Microbiol.* 56:3478–3481.
77. Snyder, J. C., B. Wiedenheft, M. Lavin, F. F. Roberto, J. Spuhler, A. C. Ortmann, T. Douglas, and M. Young. 2007. Virus movement maintains local virus population diversity. *Proc. Natl. Acad. Sci. USA* 48:19102–19107.
78. Söderström, C., H. Borén, F. Winquist, and C. Krantz-Rülcker. 2003. Use of an electronic tongue to analyze mold growth in liquid media. *Int. J. Food Microbiol.* 83:253–261.
79. Stephens, P. J., P. Druggan, and G. N. Caron. 2000. Stressed *Salmonella* are exposed to reactive oxygen species from two independent sources during recovery in conventional culture media. *Int. J. Food Microbiol.* 60:269–685.
80. Stevens, K. A., and L. Jaykus. 2004. Bacterial separation and concentration from complex sample matrices: a review. *Crit. Rev. Microbiol.* 30:7–24.
81. Stevens, K. A., and L.-A. Jaykus. 2004. Direct detection of bacterial pathogens in representative dairy products using a combined bacteria concentration–PCR approach. *J. Appl. Microbiol.* 97:1115–1122.
82. Suzuki, Y., N. Hanagasaka, T. Furukawa, and T. Yoshida. 2008. Removal of bacteria from coastal seawater by foam separation using dispersed bubbles and surface-active substances. *J. Biosci. Bioeng.* 105:383–388.
83. Tian, P., A. Engelbrektson, and R. Mandrell. 2008. Two-log increase in sensitivity for detection of norovirus in complex samples by concentration with porcine gastric mucin conjugated to magnetic beads. *Appl. Environ. Microbiol.* 74:4271–4276.

84. Verreault, D., S. Moineau, and C. Duchaine. 2008. Methods for sampling of airborne viruses. *Microbiol. Mol. Biol. Rev.* 72:413–444.
85. Viehweg, H., and K. Schügerl. 1983. Cell recovery by continuous flotation. *Appl. Microbiol. Biotechnol.* 17:96–102.
86. Vorst, K. L., E. C. D. Todd, and E. T. Ryser. 2004. Improved quantitative recovery of *Listeria monocytogenes* from stainless steel surfaces using a one-ply composite tissue. *J. Food Prot.* 67:2212–2217.
87. Wang, L. K. 2006. Adsorptive bubble separation and dispersed air flotation, p. 81–122. *In* L. K. Wang, Y.-T. Hung, and N. K. Shammas (ed.), Handbook of environmental engineering, vol. 4. Advanced physicochemical treatment processes, 1st ed. Humana Press, Totowa, NJ.
88. Wang, S., and R. E. Levin. 2006. Discrimination of viable *Vibrio vulnificus* cells from dead cells in real-time PCR. *J. Microbiol. Methods* 64:1–8.
89. Weber, M. E., D. C. Blanchard, and L. D. Syzdek. 1983. Mechanism of scavenging of waterborne bacteria by a rising bubble. *Limnol. Oceanogr.* 28:101–105.
90. Weichart, D. H., and D. B. Kell. 2001. Characterization of an autostimulatory substance produced by *Escherichia coli*. *Microbiology* 147:1875–1885.
91. White, D. C., C. A. Lytle, Y.-D. M. Gan, Y. M. Piceno, M. H. Wimpee, A. D. Peacock, and C. A. Smith. 2002. Flash detection/identification of pathogens, bacterial spores and bioterrorism agent biomarkers from clinical and environmental matrices. *J. Microbiol. Methods* 48:139–147.
92. Willson, H. S. 1905. The isolation of B. Typhosus from infected water, with notes on a new process. *J. Hyg. (Lond.)* 5:429–443.
93. Wolffs, P. F. G., K. Glencross, R. Thibaudeau, and M. W. Griffiths. 2006. Direct quantitation and detection of salmonellae in biological samples without enrichment, using two-step filtration and real-time PCR. *Appl. Environ. Microbiol.* 72:3896–3900.
94. Wong, D., and G. Robertson. 2004. Applying combinatorial chemistry and biology to food research. *J. Agric. Food Chem.* 52:7187–7198.
95. Yang, L., F. Portugal, and W. E. Bentley. 2006. Conditioned medium from *Listeria innocua* stimulates emergence from a resting state: not a response to *E. coli* quorum sensing autoinducer AI-2. *Biotechnol. Prog.* 22:387–393.
96. Yitzhaki, S., E. Zahvy, C. Oron, M. Fisher, and A. Keysary. 2006. Concentration of *Bacillus* spores by using silica magnetic particles. *Anal. Chem.* 78:6670–6673.
97. Zhang, L., Z. Yan, and E. T. Ryser. 2007. Impact of dilution ratios on *Listeria monocytogenes* growth during University of Vermont Medium enrichment of deli meats. *J. Food Prot.* 70:2656–2660.
98. Zougagh, M., and A. Ríos. 2008. Supercritical fluid extraction of macrocyclic lactone mycotoxins in maize flour samples for rapid amperometric screening and alternative liquid chromatographic method of confirmation. *J. Chromatogr. A* 1177:50–57.