
SPECIAL INTEREST

Critical Research Needs in Food Safety Microbiology

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ABSTRACT

Control of sporeforming bacterial pathogens and spoilage organisms; technology and processes to control *Salmonella* in low-moisture foods; and detection and mitigation strategies for viral causes of foodborne illness were identified as three critically important research areas by the ILSI North America Food Microbiology Committee during deliberations leading to its 2008 request for proposals (RFP). While the Committee received a number of pre-proposals in these areas, few proposals met the Committee's expectations. Therefore, the Committee is communicating the identified research needs to the larger food microbiology community to stimulate applied research, training, and funding in these priority areas. The ILSI North America Food Microbiology Committee has recently issued a 2009 RFP in one of the identified areas, "Technology and Processes to Control *Salmonella* in Low-moisture Foods." For more details on 2009 RFP for research support, please visit the ILSI North America Web page: www.ilsina.org.

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INTRODUCTION

The ILSI North America Food Microbiology Committee has a long history of funding research in the area of food microbiology and, since 1988, has issued calls for research proposals, typically every other year. Priority research areas for funding are identified after consultation with US government agencies (typically the US Department of Agriculture, US Food and Drug Administration, and the Centers for Disease Control and Prevention), food industry representatives, and academic advisors to the committee. The proposal solicitation and review process is as follows: ILSI North America issues a call for pre-proposals. The Food Microbiology Committee reviews and scores the proposals based on their relevance to the objectives defined in the RFP, and the innovativeness and scientific merit of the proposed research. Investigators who submitted highly ranked proposals are invited to submit full proposals. In 2008, the ILSI North America request for research proposals included three focus areas: Control of sporeforming bacterial pathogens and spoilage organisms, Technology and processes to control *Salmonella* in low-moisture foods, and Detection and mitigation strategies for viral causes of foodborne illness (2008 RFP). While the Committee received a number of pre-proposals in these areas, few met the Committee's objectives. The goal of this article is to communicate the critical research needs in the three areas identified by the ILSI North America Food Microbiology Committee during preparation of the 2008 RFP, proposal review, and subsequent committee discussions. The authors hope that this brief article will stimulate new research as well as funding in these important specific areas of need identified by this committee. Although this article clearly is not meant to be a complete review of all knowledge gaps in this area, it does provide a brief summary of critical research needs identified by a group of industry, government, and academic scientists with considerable expertise in food microbiology.

CONTROL OF SPOREFORMING BACTERIAL PATHOGENS AND FOOD SPOilage MICROORGANISMS

Sporeforming bacterial pathogens continue to be a major concern for public health and food safety. This concern is compounded by novel routes of transmission of these pathogens and previously unrecognized spore-forming pathogens. For example, recent cases of botulism in immunocompromised adults due to growth and toxin formation in the intestines of adult individuals with suppressed intestinal microflora (10) and of healthy children (9, 15) have raised new concerns with regard to *C. botulinum* as a foodborne pathogen. In addition, recognition of botulinum toxin-producing *Clostridium* species other than *C. botulinum* (e.g., specific *C. sporogenes* strains (14)) and foodborne toxin-producing *Bacillus* spp. other than *B. cereus* (e.g., specific *B. thuringiensis* strains (1)) represent important public health concerns. Further, sporeforming bacterial spoilage organisms also represent considerable concern for the food industry. While some sporeforming spoilage microorganisms are now well recognized and have received considerable research attention (e.g., *Alicyclobacillus* (13, 16, 17, 20)), knowledge of the ecology, physiology, and genetics of a number of sporeforming spoilage microorganisms is extremely limited (e.g., *Thermoanaerobacterium* and *Thermoanaerobacter* (2, 7)). This severely limits the ability

to (i) detect these organisms, (ii) identify sources of these organisms, and (iii) control and eliminate these organisms in the production of food products in which their presence causes considerable spoilage problems.

The ILSI North America Food Microbiology Committee identified the following research needs in the area of sporeforming pathogens and spoilage organisms:

(a) Sporeforming pathogens:

- Control of toxin formation by proteolytic and non-proteolytic *C. botulinum* in extended shelf-life foods and beverages.
- Genetics, physiology, and control of botulinum toxin-producing *Clostridium* species other than *C. botulinum*.
- Genetics, physiology, foodborne transmission, molecular subtyping and epidemiology of other pathogenic *Clostridium* spp., in particular *C. difficile*.
- Genetics, physiology, and control of foodborne toxin producing *Bacillus* spp. other than *B. cereus*.
- Association between botulinum toxin formation and organoleptic changes in foods and beverages, including an understanding of competitive spoilage microorganisms that will cause spoilage before toxin formation can occur in a given food.

(b) Sporeforming spoilage organisms:

- Novel technologies, including non-thermal treatments and combination treatments, for control of heat-resistant sporeforming spoilage organisms in food and beverage products. Examples of treatments of interest are (i) pulse electric field treatment, (ii) radiation, (iii) microwave treatment, (iv) natural antimicrobials (including bacteriocins, natural extracts, etc.), and (v) bacteriophages. Research on other truly novel interventions is also of critical importance.
- Genetics, physiology, and control of sporeforming spoilage organisms other than *Alicyclobacillus*, including *Clostridium* spp. that can cause spoilage (e.g., *Clostridium laramie*).

In regard to overarching research needs in the area of sporeformers, the ILSI North America Food Microbiology Committee identified the following:

- Control of spoilage and pathogenic sporeformers in refrigerated meals.
- Control of spoilage and pathogenic sporeformers in refrigerated extended shelf-life foods and beverages.
- Training of graduate students and new investigators in all aspects of biology, control, and transmission of spoilage and pathogenic sporeformers.

TECHNOLOGY AND PROCESSES TO CONTROL SALMONELLA IN LOW-MOISTURE FOODS

Two US outbreaks of *Salmonella enterica* subsp. *enterica* serotypes Tennessee and Typhimurium infections traced to contaminated peanut butter in 2006–2007 and 2008–2009, respectively, have once again highlighted the problem of *Salmonella* contamination of low-moisture food products (4, 5). Both were relatively large national outbreaks, and each caused illnesses in more than 500

persons. The cost to the economy of the most recent peanut butter outbreak in the US may exceed \$1 billion (18). On a global level, more than 20 foodborne disease outbreaks associated with low-moisture food products were documented in the developed regions between 1970 and 2008 (11). Foods implicated in these outbreaks included chocolate, infant cereals, milk powder, powdered infant formula, peanut butter and other peanut-containing products, raw almonds, and toasted oats cereal (12). At least four of these outbreaks occurred in the US.

Three major factors that exacerbate the problem of *Salmonella* contamination of low-moisture foods and their propensity to cause foodborne disease are the following:

- *Salmonella* cells appear to be more refractory to inactivation procedures in low-moisture foods
- *Salmonella* cells that survive inactivation treatments in low-moisture foods or that are introduced into low-moisture foods after the inactivation step through post-process contamination (more likely) may persist in these foods for long periods of time (weeks to months).
- The infectious dose for *Salmonella* in low-moisture foods may be very low (often less than 10 CFU/g), as evidenced by outbreak investigations.

Taken together, these observations lead to the conclusion that the presence of even small numbers of *Salmonella* in low-moisture foods may present a serious human health hazard and is therefore unacceptable. To address this problem, the Grocery Manufacturers Association has recently published a guidance document that identifies seven elements for the control of *Salmonella* in low-moisture foods (11, 12): prevention of introduction and/or spread of *Salmonella* in the food processing facility, enhancing the stringency of hygiene practices and controls in the primary *Salmonella* control area, incorporation of hygienic design principles in building and equipment design, preventing/controlling the growth of *Salmonella* in the processing facility, establishing a raw materials/ingredients control program, validating control measures to inactivate *Salmonella*, and establishing procedures to ensure that the *Salmonella* controls are working and for corrective actions. In March 2009, the US Food And Drug Administration issued the following recommendations to food manufacturers to address the risk of *Salmonella* contamination of foods containing a peanut-derived product as an ingredient (21):

- Purchase peanut-derived product only from suppliers who use validated processes to adequately (e.g., by 5 logs) reduce the numbers of *Salmonella* cells in their product.
- If the peanut-derived product is purchased in a form for which no validated process is available, or if there are questions concerning the presence of *Salmonella* in specific lots of peanut-derived products, ensure that the manufacturer's own manufacturing process would adequately reduce the numbers of *Salmonella* cells in their product

In regard to research needs in the area of controlling *Salmonella* in low-moisture foods, the Committee identified three specific areas and sub-topics within each area:

- (a) Persistence of *Salmonella* in low-moisture foods and processing environment:

- Define and characterize mechanisms by which *Salmonella* develops and maintains resistance to drying, including the effects of different drying processes, food matrices and strain variation.
- Identify characteristics that may allow some strains to become entrenched and resident in a dry process environment.
- Develop rapid tools to map and "fingerprint" *Salmonella* strains in low moisture food environments, allowing for differentiation between potential transient and resident strains.
- Define optimized methods for recovery and detection of desiccated *Salmonella* from dry matrices and environments.

- (b) *Salmonella* mitigation processes for use in the production of low-moisture foods:

- Generate relevant thermal death time data for a number of low-moisture food groups, and develop a model for *Salmonella* inactivation along a continuing A_w /fat spectrum; publish to a database.
- Evaluate the effectiveness of thermal processes and non-thermal process or hybrid thermal processes as pasteurization steps in the production of low moisture foods.
- Develop strategies to adequately validate these mitigation processes.
- Develop strategies to minimize *Salmonella* load in the raw agricultural commodities.

- (c) Non-aqueous sanitation processes that eliminate *Salmonella* from dry manufacturing equipment and processes, and strategies to validate the new processes.

This research funding initiative of ILSI North America Food Microbiology Committee resulted in the funding of one research project on the inactivation of *Salmonella* on raw nuts by use of low-energy X-ray. However, the Committee feels that much more comprehensive and targeted applied research needs to be conducted in this area to close knowledge gaps and to generate information that the food industry could use to control the problem of *Salmonella* contamination of low-moisture foods.

CRITICAL RESEARCH NEEDS ON DETECTION AND MITIGATION STRATEGIES FOR VIRAL CAUSES OF FOODBORNE ILLNESS

Viral pathogens, especially Norovirus and Hepatitis A virus (HAV), continue to be a major concern for public health and food safety. Both viruses are mainly transmitted from person to person, but food is also an important source when it becomes contaminated at its source from sewage, e.g., in oyster beds or produce fields, or during preparation by an ill or asymptomatic food worker. The connection to a specific food is most often apparent during outbreaks, and in the latest report on outbreaks of foodborne illness in the US states from 1998 – 2002 (3), Norovirus caused 33% of outbreaks and 41% of associated illness of foodborne infections with confirmed etiology, with an increasing trend over the period; likewise, HAV caused 2.4% of the outbreaks reported during the same period. Norovirus has been reported

to account for 25% of produce-related outbreaks (6). The infectious dose of these pathogens is small and the viruses are hardy, being stable and surviving on dry surfaces and in food for prolonged periods; they are resistant to many sanitizers, freezing and temperatures up to 60°C; they are non-cultivable, and no good animal infection models are available (8, 19). This severely limits the ability (i) detect these organisms, (ii) identify sources of these organisms, and (iii) control and eliminate these organisms in the production of food products, in which their presence is a major public health problem.

Based on the challenges outlined above, the members of the ILSI North America Food Microbiology Committee believe that considerable need exists for the food microbiology community to enhance research and teaching efforts in the area of foodborne viral pathogens. The Committee thus, in 2008, included a call for research proposals in the area of "Detection and Mitigation Strategies for Viral Causes of Foodborne Illness" in its 2008 RFP for research funding. Although the committee received eight preproposals in this area, relevance to the Committee's objectives was not met and none of the projects was funded.

In regard to research needs in the area of foodborne viral pathogens, the ILSI North America Food Microbiology Committee identified the following:

- Development and assessment of methods to detect and quantify infectious Norovirus and HAV in diverse food matrices, e.g., in seafood and produce. The method(s) should be applicable to the detection of infectious virus in foods following treatment(s) designed to reduce the risk of viral foodborne disease, such as through sanitation or processing (such as thermal processing, aseptic processing, ingredient mixing, ingredient handling, other).
- Development and assessment of methods to concentrate virus from food, e.g., by culture, filtration, adsorption, precipitation or other methods.
- Development and assessment of methods to control foodborne viral pathogens from entering the food production.
- Development and assessment of methods to control or inactivate virus from food without affecting its organoleptic quality, e.g., heat, sanitation, pressure, irradiation, other.
- Development and assessment of methods to inactivate foodborne viral pathogens from food preparation surfaces, e.g., sanitizers and disinfectants.

In regard to overarching research needs in the area of foodborne viral pathogens, the ILSI North America Food Microbiology Committee identified the following:

- Assessment of the burden of illness caused by foodborne viral pathogens.
- Development of a risk assessment for Norovirus and HAV in different food commodities.
- Training of graduate students and new investigators in all aspects of biology, control, and transmission of foodborne viral pathogens.

The Committee hopes that widespread dissemination of the research priorities identified above will stimulate researchers and investigators to focus their research in these areas and will persuade funding agencies to include these

topics in their list of priorities for funding. Additional research in these areas will lead to closing of significant knowledge gaps in food safety and will allow food processors to enhance the safety of their products and processes. The ILSI North America Food Microbiology Committee has recently issued a 2009 RFP in one of three identified areas, "Technology and Processes to Control *Salmonella* in Low-moisture Foods" (for more details visit: www.ilsina.org). The Committee may issue additional RFPs in identified research areas in the future and may consider unsolicited proposals on these topics, if they are sufficiently innovative and promising.

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