

Ontario Food Safety Research Program Compendium



2005-2006



PREFACE

Today's investments in food safety research will result in tomorrow's savings in health-care spending, industry savings and an increased ability to compete on the world stage. The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) is devoted to making the Ontario Food Safety System a world-class system to ensure that the health and safety of Ontarians comes first. For these reasons OMAFRA is dedicated to supporting state-of-the-art food safety research.

We are pleased to announce the funding of eight new research projects that will advance our knowledge about the prevalence, prevention and detection methods of food-borne hazards.

With these new projects we are investing close to \$0.6 million dollars in research projects being performed at three research institutions across Ontario, including the University of Guelph, Health Canada and Agriculture and Agri-Food Canada. This provincial investment has leveraged an additional \$0.6 million in financial and in-kind support from research partners. Furthermore, the program will be contributing to the training of highly qualified personnel by funding a total of 20 Ph.D., M.Sc. and undergraduate students.

This was the sixth year of the competitive Food Safety Research Program, and it builds on the achievements from previous years, in which it has invested \$4.7 million in 44 research projects.

The FSRP has been successful not only in attracting excellent research projects and in achieving its stated objectives, but also in fostering collaboration in food safety research, and in disseminating results of research promptly and widely.

For additional information on OMAFRA food safety research we encourage you to visit our website at: <http://www.omafra.gov.on.ca/english/research/>.

For further information on any specific projects listed in this compendium you are encouraged to contact the lead researcher directly.

Finally we would like to recognize and thank the many researchers, universities, federal and provincial government departments and industry organizations that partner with OMAFRA to fund, perform and to communicate research results. We still have a long way to go, both in terms of funding and capacity building, to ensure food safety research continues to support discovery, create new knowledge and generate innovation. Therefore, we are excited about the innovative opportunities and possibilities that have been identified and the many research institutions that have come together to define and carry on excellent, multidisciplinary food safety research. The safety of our food is very important and the research projects supported by FSRP will help enhance the safety of food for the benefit of our Ontario citizens.

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TABLE OF CONTENTS

Preface	2
Section One: Food Safety Research Program 2005/06	4
Section Two: Abstracts	7
Detection Methodology	7
Risk Assessment	11
Risk Management and Control	12
Section Three: Status of Previously Funded Projects (2000/01 - 2004/05)	15
Section Four: Quick Searches	18

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Section One: Food Safety Research Program 2005/06

In this *Compendium*, we present the outcome of the FSRP 2005/06 grant application and funding process.

For your convenience this Compendium is divided into four sections to allow greater search capabilities and ease of information dissemination.

Section One: Food Safety Research Program 2005/06

In this section you will find the program description, update, research priorities, details about the latest program competition and interesting statistical information.

Section Two: Abstracts

This section contains detailed abstracts of the projects categorized into three areas: Detection/validation methodology (DM); Risk Assessment (RA) – identification of emerging food safety hazards and contaminants; Risk Management (RM) of food safety risks. These abstracts include contact information for the lead researcher, name of collaborating researchers and project duration. In addition, for each project you will find a brief description of anticipated benefits to the food safety system in Ontario.

Section Three: Status of Previously Funded Projects (2000/01 – 2004/05)

This section provides a snapshot of all funded projects since the beginning of the program with reference to the status of the project.

Section Four: Quick Search

This section allows you to cross reference key words (e.g. *Salmonella*) throughout the supported projects.

Background

Ontario is recognized throughout the world for the quality and safety of its agri-food products. To retain this position of leadership in food safety, the province has initiated science-based, field-to-fork food safety system improvements. In partnership with the Ministry of Health and Long-term Care (MOHLTC) and the Ministry of Natural Resources (MNR), the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) led an Ontario food safety system review. During the review process, OMAFRA recognized the need to update its standards and requirements to keep pace with changes in scientific information, technology, consumer behavior, consumer lifestyles and industry practices. The review was designed to improve Ontario's food safety system by increasing the government's capacity to maintain high standards of food safety, protect public health and increase the marketability of Ontario food products. The overall goal was to build a:

- modernized, science-based food safety system founded on the principles of risk analysis/risk management;
- seamless system that covers the food chain from field to fork;
- market-friendly system consistent with Ontario's trade responsibilities and industry needs.

Program description

The *Ontario Food Safety Research Program* is a competitive research fund established in 2000/01 and funded on an annual basis. The mandate of the program is to fund innovative food safety research projects that enhance the safety of Ontario's food through:

- Development and/or validation of testing methods for the detection of pathogens and chemicals for use in laboratory and field settings;
- Identification of emerging food hazards and contaminants;
- Risk analysis, risk assessment, risk management and control in food safety.

Ultimate results of the program are new and/or enhanced technologies and diagnostic tools that support the agri-food industry and government regulatory and laboratory programs; new knowledge about emerging food hazards and contaminants; new strategies to reduce, eliminate and manage food safety risks. These results contribute to and support the implementation of Hazard Analysis Critical Control Point (HACCP) and quality assurance programs throughout the food chain.

A Research Requirements Document is issued on an annual basis to solicit research proposals from academia, industry, and government or partnership networks with demonstrated capability to perform quality research in their area of expertise. For the 2005/06 competition the researchers were eligible to apply for up to \$100,000 per project that has to be completed within two years. The program strongly encourages the applicants to demonstrate extensive collaboration and secure matching funding if possible. OMAFRA staff and external peer reviewers review submitted proposals. Under the 2005/06 competition we received 41 Letters of Intent in response to our June, 2005 Call for Proposals.

The full abstracts of these newly funded projects, as well as those from previous FSRP funding cycles, can be found on our Web site:

<http://www.omafra.gov.on.ca/english/research/foodsafety/2005/index.html>

With these new projects we are investing almost \$0.6 million dollars for research being performed at three research institutions across Ontario, including the University of Guelph, Health Canada and Agriculture and Agri-Food Canada.

It is important to highlight that from a total investment of \$0.6 million 90% goes directly to support research and only 10% is for administrative costs.

To be successful in obtaining the program funds the researchers must satisfy the following program criteria:

- Fit to the research priorities described in this document; the relevance to current issues in food safety; the anticipated contribution to improving the food safety system in Ontario
- Quality and clarity of experimental design and project work plan
- Research capabilities of the researcher and establishment
- Contribution from collaborators and the impact on the quality of research attained
- Appropriateness of budget items - project costs must be reasonable and detailed
- Effectiveness of the technology transfer and communications plan in facilitating the adoption or commercialization of the research results

Overall the project proposals should:

- Foster innovative ideas (i.e., new detection methods, new strategies to reduce, eliminate, manage food safety risks)
- Nurture collaboration and synergy between food safety scientists, government agencies, policy makers and the industry
- Complement, build on, and/or feed into, but not duplicate the research programs of other funding agencies interested in food safety
- Encourage multi-disciplinary, collaborative participatory research
- Allow researchers to explore speculative 'high reward' opportunities
- Bring new researchers into food safety research
- Encourage special topics not well covered by other funding agencies

Statistical Summary:

Overall FSRP Funding in 2005/06

- Letters of Intent received = 41
- Letters of Intent invited to submit full proposal = 17
- Applications offered funding = 8
- Success rate = 19.5%

Applications and Awards by FSRP Priority Area:

Priority Area	# Applications	#Grants Awarded
Development and Validation of Testing Methods (DM)	19	4
Risk Assessment (RA)	6	1
Risk Management (RM)	16	3

FSRP 2005/06 Funding Highlights

- Four awarded projects focus on innovative, exploratory, and high-risk/high reward research projects in development of a novel approach in detection methodology by focusing on development and/or validation of biosensors, microarrays and PCR methodology in the detection of food-borne viruses, mycotoxins and bacteria.
- One awarded project focuses on risk assessment – specifically on the assessment of cost-effectiveness of alternative measures for reducing the prevalence of food-borne microbiological hazards in Ontario.
- Three awarded projects deal with innovative approaches to eliminate, reduce and manage food safety hazards, such as use of bacteriophages to destroy *Salmonella* in pork production, use of antimicrobial photodynamic treatments for surface sanitation and use of effective enzyme products to degrade mycotoxins in contaminated grains.

The FSRP wishes to acknowledge and thank the peer reviewers who participated in the program review process for their service and dedication to the Program.

Section Two: Abstracts

Detection Methodology

DM

DM1: An Integrated System for the Detection and Characterization of Enteric Viruses in Ready-to-Eat Foods

Abstract:

Enteric viruses are responsible for $\geq 67\%$ of foodborne diseases in the US, and are increasingly recognized as important threats to public health. Recently, Noroviruses (NoVs) have become the number one virus involved in foodborne outbreaks. Other important foodborne viruses include hepatitis A viruses (HAVs) and rotaviruses (RVs). Methodologies for the isolation and detection of these viruses from foods lag severely behind their bacterial counterparts. Most contaminated foods contain few viral particles and viruses cannot grow outside of their host; viruses must therefore be concentrated from foods prior to detection. Existing isolation procedures are lengthy, technically difficult to perform, not universally applicable to food matrices, and can damage the integrity of virus particles. Currently, viral nucleic acid amplification by RT-PCR is the most widely used method for virus detection. The development of a universal, rapid and easy-to-use method for the isolation and identification of foodborne viruses is important to ensure the safety of the Ontario food supply. This would reduce the time and cost required for sample analysis by allowing laboratories to adopt a single, simple method. This study aims to develop a rapid and universal viral concentration method, coupled to a DNA microarray platform for simultaneous identification and genotyping. Virus concentration from foods will be achieved using the Pathatrix™ system. Multiplex PCR will be standardized for the amplification of all viruses of interest and the Norochip (OMAFRA 2003-2005) will be expanded to allow identification and genotyping of HAV, ADV and RV. This universal approach to the identification and characterization of foodborne viruses will allow for quicker response to potential contamination events, more informed control efforts, and better risk assessment and policy-making decisions to control the spread of foodborne viral diseases.

Expected Impact of Project Outcomes on Food Safety in Ontario:

A recent study in the Hamilton region estimates the cost burden of gastrointestinal disease at CDN\$115 per person per year, or over \$1.4 billion dollars annually for the province of Ontario. Viral agents are responsible for a large percentage of this gastrointestinal disease. While epidemiological data implicate food as a major vehicle of virus transmission, our ability to rapidly and accurately detect viruses in food remains limited. As a result, the source and epidemiology of viral outbreaks cannot always be pinpointed and reported. Our goal is to develop the Pathatrix™ system into a universal method for the isolation of foodborne viruses from different food matrices and to expand the DNA microarray method pioneered in our laboratory for detecting norovirus. We will integrate virus capture technology into the existing protocol and improve our microarray design to allow detection and typing of additional foodborne viruses. This will result in a standardized two-step system for the isolation, detection and simultaneous genotyping of norovirus, rotavirus, hepatitis A virus and other enteric viruses. This system will generate a single protocol to screen for multiple virus types, saving time and money over current methods which lack standardization and may not be applicable to different types of foods or viruses. The development of this detection system should provide Ontario with the best and latest technology to screen for viruses in foods, thus improving detection and assessment of viral contamination of food and water in Ontario. The epidemiological data obtained from virus typing will be useful in future risk assessment studies. These tools should provide us with the means to improve the quality and safety of foods in Ontario.

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DM2: Detection and Quantification of Pathogenic Viruses in Ready-To-Eat (RTE) Foods

Abstract:

Data from the Centre for Disease Control and Prevention (CDC) published in 1999 suggests that almost 80% of the estimated 38.5 million illnesses due to foodborne pathogens annually in the US are attributable to viruses. Of these the vast majority (23 million cases) are caused by noroviruses with hepatitis A accounting for a further 83,400 cases. Very little is known about the prevalence of viruses in our food supply although the major sources of these viruses appear to include foods such as shellfish, fruits and vegetables coming into contact with contaminated water in their growing area before harvest; and foods that become contaminated during preparation through contact with fecally contaminated surfaces or infected food handlers. Despite these high figures, methods to detect foodborne viruses are still in their infancy but with the advent of molecular techniques such as PCR, reliable and rapid assays are emerging. However, one of the major problems in the application of PCR protocols to foods is the removal of target genetic material in a form that can be amplified. To remove and concentrate targets from food, methods such as immunomagnetic separation and filtration have been applied but work to date has mainly focused on bacterial pathogens. Also PCR assays provide an opportunity to detect more than one organism in a single sample, but, again, this multiplexing approach has not been widely used in the field of food virology.

This study will build on research that has been completed in the Canadian Research Institute for Food Safety (CRIFS) laboratories in which has developed a real-time RT-PCR assay for the detection of hepatitis A in fresh produce. The aim of this project is to develop a real-time PCR methodology for detection and quantification of pathogenic viruses in Ready-to-Eat (RTE) foods, wherein the main focus will not only be the development of a multiplex quantitative real-time PCR assay but especially the development of a sample treatment method capable of concentrating very low amounts of virus from the food sample. To achieve this we will study immunomagnetic separation, filtration using charged membranes and a proprietary product produced by a Canadian company, EcoVu Analytics, which uses novel chemistry to capture particles from suspension, to capture and concentrate virus particles from a variety of foods.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Noroviruses (NV) and hepatitis A virus (HAV) are the most commonly reported food-borne viruses worldwide with NV estimated as the leading cause of food-borne illness in the US. In Ontario about 50% of the hepatitis A reported cases are associated with food- or water-borne transmission. It has been estimated that the costs of hepatitis A infections may be as much as \$3,000 per adult case and \$1,750 for infant cases. Health-care associated outbreaks of gastroenteritis are also becoming an increasing problem. A study in the UK showed that NV was responsible for 63% of such outbreaks and gastroenteritis outbreaks as a whole were estimated to cost the English National Health Service a staggering \$2.5 million each year.

Common-source outbreaks are frequently due to contamination of the food by an infected food-handler, just prior to consumption. However, reports of contamination of foods before retail distribution are increasing and may be prevented by appropriate testing of high-risk food commodities. The aim of this project is to use real-time RT-PCR for rapid simultaneous detection and possible quantification of NV and HAV in RTE foods. To obtain efficient detection, it is crucial to integrate a real-time PCR method with a suitable sample preparation method. Therefore, methods to concentrate possibly very low amounts of virus before PCR detection and remove PCR inhibitors have to be developed or improved. Finally, by developing a quantitative method, much needed data on infectious dose and virus contamination levels in RTE foods will provide information to OMAFRA which will be invaluable in setting priorities for provincial food safety programs.

DM3: Mycotoxin Detection in Foods Using Electrochemical Sensors Based on Affinity Molecules Incorporated into Conducting Polymer Films

Abstract:

Mycotoxin is a broad term to describe toxic secondary metabolites derived from certain filamentous mold strains (*Aspergillus*, *Penicillium*, *Fusarium*). Any crops (pre- or post-harvest) that support the growth of molds can potentially become contaminated with mycotoxins, although cereals (wheat, barley, maize, rye) and oilseeds are considered high risk commodities. A common feature of mycotoxins is the high binding affinity to DNA and proteins leading to chronic conditions such as cancer, immuno-suppression or organ damage. Mycotoxins are highly stable and can accumulate within the body when ingested in small quantities over prolonged time periods. In animals (especially pigs), the production period is typically too short to result in chronic toxicity, however, even moderate levels can detrimentally affect their development.

Due to the stability of mycotoxins there is no reliable method to inactivate or remove the toxic agent should contamination occur. Therefore, there is a high reliance on sample screening to identify contaminated batches and restrict entry into the food chain. Because mycotoxins represent a hazard even when present in trace amounts, regulatory limits are set in the parts per billion (ppb) and sample sizes are typically large (0.1-1 kg). Therefore, this necessitates initial extraction and concentration of mycotoxins from samples prior to detection using techniques such as HPLC or ELISA. Current methods are laboratory based, require a high level of expertise and are time consuming. An ideal alternative would be to screen for mycotoxins on-site thereby providing rapid results and immediate corrective action to take place (withdrawal or diversion of contaminated batches). Current on-site tests based on ELISA dip-sticks have complicated protocols and poor sensitivity. In the following project a self contained rapid, on-site, screening method that enables extraction, capture and detection of mycotoxins with minimal user input will be developed. Mycotoxins (aflatoxin B1, ochratoxin A, fumonisin B1, DON and patulin) will be extracted into aqueous solutions of methanol and concentrated (captured) via passage through molecular imprinted conducting polymer films.

The mycotoxin in the eluent will be injected into a flow injection system and passed over a conducting polymer modified electrode containing a binding affinity agent (antibody, nucleic acid, serum albumin). The binding of mycotoxin to affinity agent will be reagentless detected through monitoring changes induced in the electrochemical properties of the supporting conducting polymer film. It is envisaged that the sensor will have a lower detection limit in the range of ppb.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The mycotoxin levels in grains (wheat and corn) within Ontario are amongst the highest encountered within Canada. This is primarily due to the climatic conditions within the province that are conducive to mycotoxin formation by contaminating molds.

Due to the inherent stability of mycotoxins there is a strong reliance on screening methods to prevent contaminated products from entering the food chain. This not only includes foods destined for human consumption but also animal feed since mycotoxins can be readily transferred via meat, milk, cheese and eggs. Currently, the majority of screening is performed within commercial laboratories at a cost of \$18-125 per test and turn-around time of up to 7 days. Therefore, any action taken is retrospective.

The system to be developed will enable detection of ppb levels of mycotoxins within 30 minutes with minimal user input. On-site testing will facilitate more frequent screening for mycotoxins, but more importantly, enable corrective action to be taken should contaminated batches be detected. The system to be developed can be fully automated and enable detection of multiple mycotoxin types simultaneously. It is envisaged that the output of the research will provide a powerful tool to reduce mycotoxins within the food chain and further enhance food safety standards within Ontario.

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DM4: Concentration and Detection of Pathogens in Bean Sprout Spent Irrigation Water using Microfiltration Coupled with Electrochemical Immuno-sensors

Abstract:

Despite the implementation of microbiological safety guidelines in the latter part of the 1990's there continues to be sporadic outbreaks of foodborne illness linked to contaminated sprouted seed. Part of the guidelines recommended screening spent irrigation water for *Salmonella* and *Escherichia coli* O157:H7 48h into sprout production, thereby preventing any contaminated product being released to market. However, research by the applicant has established that contamination within sprouting mung bean beds is heterogeneously distributed. Hence, relying on a single spent irrigation water sample, as recommended, does not provide a reliable index for assessing the microbiological status of the sprouting seed bed. In commercial terms, testing multiple spent irrigation water samples from a single seed bed would be economically unfeasible. A more practical approach would be to test a single composite sample of spent irrigation water collected from different points from under the sprouting seed bed. However, by compositing large sample volumes it is possible that contamination present would be diluted and unlikely to be detected using conventional microbiological methods.

The aim of this work is to develop an integrated, on-site system that permits concentration of target pathogens from large sample volumes (10 liters) and detection via electrochemical based immuno-sensors. Initial concentration of pathogens (*Salmonella* and *E. coli* O157:H7) from spent irrigation water (10 liters) will be achieved by initial pre-filtration using a 10 μ m pore size membrane filter followed by circulation through a tangential cross-flow membrane filter (0.2 μ m pore size). This will result in a 100 fold concentration of bacteria within the spent irrigation water. The retentate from the tangential filtration step (100 ml) will be introduced into a stream of carrier solution and pathogens captured by antibodies immobilized on the surface of a modified electrode. Detection of bound cells will be achieved through addition of a secondary enzyme labeled antibody or via detecting the metabolic activity of the bound cells (oxygen consumption, free radical formation, alkaline phosphatase, nitrite reductase, urease activity). Electrodes will be overlaid with permselective membranes to minimize non-specific binding events and facilitate selective amperometric detection of the electroactive species. Under optimized conditions the recovery of target cells will be close to 100% with the lower detection limit for the electro-immuno sensor being 100 cfu/ml.

The project brings together research scientists from the U.S. FDA and Guelph University with extensive experience in the microbiological safety of sprouted seeds. Major sprout producers within Ontario and the U.S. will also participate in the project.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The sprouted seed market within Ontario is valued at >\$4m with more than 10% of Ontarians consuming sprouts on a regular basis. The popularity of sprouts can be attributed to the unique sensory properties and high nutritional content (high anti-oxidant, anti-carcinogens, anti-cholesterol constituents). However, health authorities cannot promote the consumption of raw sprouted seeds due to the inherent food safety risks associated with such products. This was underlined, by the major outbreak of salmonellosis within Ontario in 2005 that was subsequently traced to contaminated mung bean sprouts. In total, there have been 8 outbreaks (725 cases) and several product recalls of suspected contaminated sprouted seed in North America over the last 2 years. Therefore, more effective methods are required to remove (e.g. seed decontamination) or detect contamination in the course of sprout production. The key benefits of the proposed project will be to provide a technology that can both concentrate and detect contamination (if present) within large volumes (10 liters) of composite spent irrigation water in a semi- or fully automated rapid (4 h) process. This will facilitate on-site testing enabling the sprout producer to screen spent irrigation water later into the sprouting process when pathogen levels (if present) will be high. The initial cost of the unit is estimated at \$4,000. The cost per sample (taking into account regeneration of membrane and sensor interface) will be \$10-40. Although the current application is focused on sprouted seeds the technology will find utility in other sectors where detecting low levels of pathogens in large sample volumes is required (e.g. water, fresh produce testing).

Risk Assessment

RA1: Assessing the Cost-Effectiveness of Alternative Measures for Reducing the Prevalence of Food-borne Microbiological Hazards in Ontario

Abstract:

This project aims to contribute to the enhanced cost-effectiveness of interventions aimed at reducing the incidence of food-borne microbiological hazards through the food supply chain in Ontario and thus contribute to the more effective use of scarce resources in the control of food-borne illness by developing, validating and applying an economic cost-effectiveness framework. The project brings together expertise and on-going research by the Principle Investigators, OMAFRA and other government agencies to identify cost-effective interventions for reducing levels of contamination in priority pathogen/food contexts, taking an entire supply chain approach. Moreover, it will develop a framework that can be used by OMAFRA and other government agencies in developing and implementing measures aimed at the control of pathogens through food supply chains more generally. The project involves the construction of frameworks for assessing the cost-effectiveness of alternative interventions for the control of pathogens in at least two pathogen/food contexts, selected on the basis of consultation with OMAFRA and other provincial and federal government agencies. The framework will consist of three inter-related stochastic simulation models that represent the level and flow of pathogens through the commodity supply chain, impact on pathogen levels of alternative interventions and the costs associated with each intervention. These models will be constructed on the basis of the existing literature and consultation with a wider body of experts, and validated through a workshop of experts and interested parties. The models will be estimated employing data derived from existing research, a Delphi survey of experts and enterprise-level case studies. Having derived the requisite data, simulations will be undertaken to estimate the cost-effectiveness of alternative interventions and their sensitivity to model parameters. These will be validated and their policy implications assessed through a workshop involving experts and interested parties. Dissemination and communications will be undertaken throughout to ensure effective engagement of interested parties in both the public and private sectors.

Expected Impact of Project Outcomes on Food Safety in Ontario:

As part of its on-going efforts to promote the safety of the food supply in Ontario, aimed at enhancing public health, managing the associated costs of food-borne illness and promoting the competitiveness of the agri-food sector, OMAFRA is exploring ways in which control measures can be enhanced. In the context of inevitable resource constraints, it is imperative that interventions employed along the supply chain to reduce levels of contamination are cost-effective; they achieve significant reductions in pathogen levels when applied in practice for the resources required to be invested. Assessing the cost-effectiveness of alternative control measures is made more complex by the shift in focus towards an entire supply chain or 'farm to fork' perspective, requiring that potential interventions at all levels of the supply chain are examined, both individually and in combination. Such measures might include, for example, the adoption of HACCP at the farm production and/or processing stages, implementation of specific production and/or processing controls, application of good agricultural and/or manufacturing practices, etc. Thus, action might be required (and costs imposed on) both the public and private sectors. Further, actions at one level of the supply chain will impinge on the need for action at another stage, with significant distributional implications. The research proposed here aims to develop and apply a framework that will assist in assessing the cost-effectiveness of alternative interventions, in the context of priority pathogen/food combinations that have been judged to be of high priority. In so doing, the research will contribute to the enhancement of food safety in Ontario, ensuring that available resources are employed in a manner that achieves optimal reductions in levels of contamination and, at the same time, promotes the economic competitiveness of the agri-food sector in the province.

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RM1: Antimicrobial Photodynamic Treatment for Surface Sanitation

Abstract:

Certain non-toxic dyes (photosensitizers), when illuminated with visible light, can produce short-lived cytotoxic species that possess antimicrobial activity. The term Antimicrobial Photodynamic Treatment (APDT) has been adopted to describe this process. Our preliminary results show that APDT is effective against such important food-borne pathogens as *Escherichia coli* O157:H7 and *Listeria monocytogenes*, and, recently, we have shown that APDT can also be successfully applied for inactivation of microorganisms present in biofilms. These features make APDT a promising approach to be explored for the disinfection of food contact surfaces.

Another feature which makes APDT an appealing technology is that development of resistance to APDT has not been reported, which is not the case with commonly used sanitizers. In addition, sanitizers currently used by the food industry very often are not effective against bacterial spores, biofilms, or viruses. This increases the need to develop novel cost-effective strategies for surface decontamination and sanitation that will decrease bacterial loads of ready-to-eat foods, improve shelf-life, decrease possible economic losses due to spoilage and recalls, and, ultimately minimize the risk of infections.

Although clinical applications of APDT, including combating infections in wounds and burns, infections in body cavities (such as the mouth, ear, sinus, and stomach) and surface infections of cornea and skin, are being investigated, the efficacy of this method for surface disinfection has not been investigated. This project's research aims at assessing the effectiveness of a wide range of photosensitizers, including those described to be effective in clinical studies as well as new ones, for decontamination of surfaces composed of different materials relevant to the agri-food industry such as plastics, stainless steel, etc. The efficacy of the treatment will be optimized in terms of photosensitizer concentration, time of treatment and intensity of light necessary to initiate an effect. The possibility of creating self-decontaminating surfaces by incorporation of the chosen photosensitizer into suitable materials will be explored.

The objective of this investigation will be the development of new surface sanitation techniques for use in the agri-food industry. This can lead directly to improvement of health of animals and safer food products.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Numerous outbreaks of foodborne illness have been attributed to post-process contamination of product due to inadequate sanitation of food contact surfaces. As well as reducing the burden of illness, more effective sanitation protocols will lead to substantial savings to governments and companies, especially those exporting to the U.S., by reducing the number of outbreaks and recalls of meat products. Since January 2003, there have been 14 recalls of meat products in Canada; three due to contamination by *Listeria monocytogenes* and 11 caused by *E. coli* O157:H7. Thus, the development of novel, cost-effective strategies for minimizing pathogenic contamination of ready-to-eat foods is of significant importance. Major routes for secondary contamination are processing surfaces and utensils. Commonly used sanitizers very often are not effective against bacterial spores and biofilms. Besides, recent research has indicated that pathogens can acquire resistance to sanitizers and, as a result of such adaptation, cross-resistance to antibiotics has been observed. Emergence of multi-antibiotic resistant pathogens is a risk to animal and human health as well as to the safety of food products. Development of resistance to Antimicrobial Photodynamic Treatment (APDT) has not been reported, which makes this approach more attractive for investigation. Implementation of the proposed new approach for surface sanitation will result in an improvement to the quality of food as well as a decrease in possible economic losses due to spoilage and recalls.

RM2: Bacteriophage Based Interventions to Reduce the Dissemination of *Salmonella* in Pork Production, During Transportation and Holding

Abstract:

The carriage of *Salmonella* on pork continues to represent a key food safety issue. Through various lines of research it has become established that the pig environment, both on the farm and prior to slaughter (transporters and holding areas), can contribute significantly to the carriage of *Salmonella*. Such environments are difficult to sanitize and any benefits derived from increased sanitation are only short-lived due to rapid re-contamination by pigs. In the current project, the potential of bacteriophage (viruses that infect bacteria) to control *Salmonella* within pigs and their environment (farm, transporter and holding area) will be evaluated. The key benefits derived from bacteriophage include specificity to target host, self-perpetuating, self-limiting, stable, low-cost and non-toxic effects towards eukaryotes.

Baseline studies will isolate phage from samples derived from farms and processing environments. The proportion of lytic and lysogenic phage recovered from samples will be correlated to the genetic structure of *Salmonella* populations in terms of temporal stability and diversity. This will provide valuable knowledge on the role of bacteriophage in defining the persistence of *Salmonella* within the pig environment. More significantly, the results will enable an assessment to whether *Salmonella* exposed to bacteriophage develop resistance or if different (non-susceptible) genotypes become established. The latter can be addressed through using an appropriate combination of bacteriophage.

Candidate bacteriophage exhibiting strong lytic activity and broad host range will be taken forward for further study. Here the stability and replication of bacteriophage under different environmental conditions (temperature, host cell density; phage:host cell ratio (MOI)) will be assessed. In addition, the ability of bacteriophage to replicate under simulated gastro-intestinal tract conditions will be determined. Bacteriophage exhibiting high environmental stability and ability to replicate under sub-optimal conditions will be evaluated as a *Salmonella* biocontrol method using animal and environmental models.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Evidence to date identifies the pig environment as the most significant source of *Salmonella*. Here, the pathogen not only can become disseminated within herds but also passed onto successive groups of pigs raised in the same environment.

The epidemiology of *Salmonella* within pigs and their environments is not well understood. On certain farms the pathogen can have a transient existence whilst on others the bacterium can become endemic. The role of bacteriophage in defining the persistence of *Salmonella* has not been considered to any great extent. The hypothesis of the current study is that farms exhibiting low prevalence of *Salmonella* harbor virulent bacteriophage that prevent endemic populations of the pathogen becoming established. If true then this will provide an effective, easy to apply (spray) method, for controlling *Salmonella* throughout the pork chain. Key to the success of bacteriophage based control strategies is selection of the appropriate cocktail of phages with a sufficient broad host range, environmental stability and method/frequency of application. Such factors will be studied in detail in the proposed work.

The output of the research will provide a greater understanding on the role of bacteriophage on the population dynamics of *Salmonella* associated with pigs. The study will also provide a relatively simple control strategy to ultimately reduce the carriage of *Salmonella* on pork carcasses.

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Project Duration:
2006 - 2008

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RM3: Development of an Effective Enzyme Product to Degrade Trichothecene Mycotoxins in Contaminated Grains

Abstract:

Mycotoxin contamination of food and feed ingredients has been an ongoing and serious threat to human health as well as to food and livestock industries. However, preventing food or feed contamination by mycotoxins is not always feasible or practical. Therefore developing and implementing innovative mycotoxin detoxification methods are essential in food safety management, and are particularly important in Ontario where grain production and cereal-based food industries are significant agricultural sectors. This research will focus on the development of an enzyme product that is able to effectively degrade and thus detoxify trichothecene mycotoxins, including deoxynivalenol (DON or vomitoxin) and T-2 toxin, by transforming them into compounds several hundred times less toxic. In an ongoing research project, supported by both Agriculture and Agri-Food Canada (AAFC) and Ontario Pork, our multidisciplinary team has successfully identified several bacterial isolates that can completely transform trichothecene toxins into much less toxic forms. In this OMAFRA supported project, the trichothecene-transforming bacterial isolates will be characterized for their capability to produce trichothecene degrading enzyme(s). The factors affecting growth of the bacterial isolates, including culture conditions, growth rate and requirements, and utilization of nutrient sources, etc. will be examined. The efficiency of trichothecene-degradation by the bacterial isolates and the location of enzyme activity will also be determined. The promising bacterial isolates will subsequently be tested in scale-up fermentation experiments. A strategy for isolation of trichothecene-degrading enzyme(s) will be developed upon determining the characteristics of enzyme activity, which will include the use of a variety of biochemical and chromatography techniques. The enzyme product obtained from these studies will be evaluated for its usefulness in trichothecene mycotoxin degradation in food and feed ingredients. The success of this project will deliver: i) an effective enzyme product capable of degrading trichothecenes at a laboratory scale; ii) essential data for future development of commercial enzyme products; and iii) potential identification of the trichothecene degrading gene.

Expected Impact of Project Outcomes on Food Safety in Ontario:

DON and T-2 toxin are members of the trichothecene family, one of the most important mycotoxin groups, and are very frequently encountered in human foods, e.g. corn meal and granola. The presence of mycotoxins in food designated for human consumption is a food safety hazard and threat to human health. In Canada, when mold-infested corns and other grains such as wheat and barley are brought to a country elevator, they will normally be downgraded or rejected. In 2003, DON was detected in 63% of samples of cereal-based infant foods from the Canadian retail market. Ontario produces about 200 million bushels of grain corn annually and mycotoxin contamination remains a major challenge facing the industry. During 1980-1995, DON occurrence in Ontario corn varied from 12.5% to 100%, and the levels ranged from 0.02 to 4.09 ppm. Preventing mycotoxin contamination by pre-harvest management, including breeding for resistant varieties and use of fungicides, has not been efficacious in eliminating mycotoxins from food and feedstuff. It is expected that progress in the control of mycotoxin contamination will depend on the introduction of technologies for specific and efficient detoxification. Because trichothecene toxins are resistant to high cooking temperatures and chemical and physical degradation methods, biological detoxification has become a more promising approach. Our research team has successfully identified and isolated several bacterial isolates that can effectively detoxify trichothecene toxins through degradation. Further this project will identify and produce the degrading enzyme(s) from the bacteria so that they can be used to reduce/eliminate mycotoxins in grains and as an additive in animal feed. The project will also characterize the enzyme to enable future transgenic approaches to be developed for trichothecene degradation.

SECTION THREE: Status of previously funded projects (2000/01–2004/05)

The details about previously funded projects by the program are available on our website: www.omafra.gov.on.ca/english/research/foodsafety/

Lead Researcher	Project Title	Status
Detection Methodology (DM)		
Dr. Archambault Marie, University of Guelph	Johne's Disease – New Test Validation	Completed
Dr. Bidawid Sabah, Health Canada, Ottawa	Development of a Rapid Microarray Diagnostic Assay for Detection of Norwalk-like Viruses in Food	Completed
Dr. Brown Stephen, Queens University	Development of a Rapid, Sensitive and Reliable Test for the Detection and Quantification of <i>Escherichia coli</i> in Foods	Completed
Dr. Chen Shu, University of Guelph	Improvement and Validation of a DNA Microchip-based Test for Rapid and Simultaneous Detection of Six Food-borne Pathogens in Food Samples	Completed
Dr. Chen Shu, University of Guelph	Development of a Novel Protein Chip-based Test for Rapid and Cost-effective <i>Salmonella</i> Serotyping	Completed
Dr. Chen Shu, University of Guelph	Development of a Robust DNA Preparation Method to Enhance Simultaneous Detection of Multiple Pathogens in Foods by a Microarray-based Assay	Completed
Dr. Chen Shu, University of Guelph	Improvement and Validation of a Novel Protein Microarray Assay for <i>Salmonella</i> Serotyping	Ongoing
Dr. Griffiths Mansel, University of Guelph	New Technologies for Improving Real-time PCR Methods for Detection of Food-borne Pathogens	Completed
Dr. Griffiths Mansel, University of Guelph	Rapid Phage-based Method for the Detection of Pathogens in Food	Completed
Dr. Gyles Carlton, University of Guelph	Validation of a Method for Determining the Species of Origin of Contaminant <i>E. coli</i>	Completed
Dr. Hall Christopher, University of Guelph	Fluorescence Polarization Immunoassays (FPIA) for Food Safety: a Rapid Detection System for Pathogens and Chemicals	Ongoing
Dr. Ismail A.A., McGill University	Rapid Whole-Organism Identification Methods Based on Fourier Transform Infrared (FTIR) Spectroscopy	Ongoing
Dr. Krull, Ulrich University of Toronto	Rapid Ultra-Concentration Technologies for Isolation of Pathogen DNA and RNA Markers from Foodstuffs for On-line Detection and Screening	Ongoing
Dr. McEwen Scott, University of Guelph	<i>Campylobacter</i> Isolation Methodology and Molecular Characterization	Completed
Mitchell Mark, University of Guelph	Development and Validation of a Screening Protocol for Identifying Sulfamethazine-violative Swine Carcasses at Ontario Abattoirs Using the CHARM ROSA™ Sulfamethazine Test	Completed
Dr. Mutharia, Lucy University of Guelph	<i>Mycobacterium Avium</i> Subsp. Paratuberculosis: Novel Tools to Identify Risk for Contamination of Foods and Environment	Ongoing
Dr. Odumeru Joseph, University of Guelph	Application of the Impedance and Colorimetric Systems for Rapid and Cost Effective Detection of <i>Listeria</i> Species in Food and Environmental Samples	Completed
Dr. Odumeru Joseph, University of Guelph	Evaluation of Immunoassay Based Kits with High Sensitivity and Specificity for Rapid Detection of <i>E. coli</i> O157:H7 in Foods	Completed
Dr. Schraft Heidi, Lakehead University	Detection of <i>Campylobacter jejuni</i> by Fluorescent <i>in Situ</i> Hybridization	Completed
Spilsbury Louise, University of Guelph	Development of an Analytical Method for the Confirmation of Sulfonamides in Animal Tissues	Completed

Lead Researcher	Project Title	Status
Dr. Warriner, Keith University of Guelph	Reagentless Impedimetric Biosensors for Detection of Pathogens in Greenhouse Operations and Pork Processing	Ongoing
Risk Assessment (RA)		
Dr. Allan, Brenda University of Saskatchewan	Identification of Bacterial Components that Influence Colonization of Poultry by <i>Campylobacter jejuni</i>	Completed
Dr. De Lange, C. F. M., University of Guelph	Liquid Feeding of Swine – Potential Positive and Negative Impacts on Pork Safety	Completed
Dr. Friendship, Robert University of Guelph	Surveillance of Ontario Pig Farms for Diseases of Public Health Significance	Completed
Dr. Griffiths, Mansel University of Guelph	Investigation of Routes for Transfer of Food and Water-borne Pathogens to Produce	Completed
Dr. Holley, Richard University of Manitoba	Evaluation of Pesticide Solutions in the Transmission of Pathogenic Bacteria to Horticultural Crops	Completed
Dr. Kelton, David University of Guelph	Assessing the Incidence of Antimicrobial Resistant <i>E. coli</i> and <i>Salmonella</i> Bacterial Isolates in Cull Cows from Ontario Free Stall Dairy Herds	Completed
Dr. McEwen, Scott University of Guelph	Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Poultry Products and Human Cases in a Southwestern Ontario County	Completed
Dr. McEwen, Scott University of Guelph	A Novel Evidence-based Tool in Support of Food Safety Policy Development	Ongoing
Dr. Pollari, Frank P.H.A. of Canada	Prevalence and Enumeration of Food-borne Microbial Hazards in Retail Raw Meat Products in an Ontario Community	Ongoing
Dr. Ribble, Carl University of Guelph	Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Turkey and Veal Products in Southwestern Ontario	Completed
Dr. Warriner, Keith University of Guelph	Establishment of Critical Control Points for Enteric Pathogens in Beef Production	Ongoing

Risk Management (RM)		
Dr. Abernathy, Tom McMaster University	A Community Trial to Determine an Effective Intervention for the Delivery of HACCP to the Food Service Sector	Completed
Dr. Friendship, Robert University of Guelph	Control of <i>Salmonella</i> and Other Pathogens of Public Health Concern on Ontario Pig Farms	Ongoing
Dr. Gong, Joshua AAFC, Guelph	Evaluation of Essential Oils as an Alternative to Dietary Antibiotics to Control Food-borne Pathogens in Livestock	Ongoing
Dr. Henson, Spencer University of Guelph	Understanding Barriers to the Effective Implementation of HACCP in the Ontario Food Processing Sector	Completed
Dr. Johnson, Roger P.H.A. of Canada	Bacteriophage Therapy to Control <i>E. coli</i> O157:H7 in Cattle	Ongoing
Dr. McAllister, T. A. AAFC, Lethbridge	Evaluation of the Ability of Seaweed Extract (Tasco-14) to Reduce the Duration and Intensity of Fecal Shedding of <i>Escherichia coli</i> O157:H7 and Total <i>E. coli</i> by Cattle	Ongoing
Dr. Sharif, Shayan University of Guelph	Antibiotic Replacement Therapy for Control of Food-borne Pathogens in Poultry	Ongoing
Skinner, Alison Ontario Beekeepers' Association	Enhancing the Food Safety of Honey Bee Hive Products through the Use of Organic Beekeeping Practices and Effective Monitoring of Pest and Disease	Completed
Dr. Szymanski, Christine National Research Coun.	Reduction of <i>Campylobacter jejuni</i> Colonization in Poultry	Ongoing
Dr. Warriner Keith, University of Guelph	Elimination of Human Pathogens on Seeds Destined for Sprout Production Using a Novel Sanitizer	Completed
Dr. Warriner Keith, University of Guelph	Ultra Violet and Hydrogen Peroxide Combination for Decontaminating Minimally Processed Fruits and Vegetables	Ongoing
Dr. Zhou H., University of Guelph	Practical Optimization of Ozonation Process for Enhancing Microbial Safety and Food Quality	Completed

SECTION FOUR: QUICK SEARCH

Subject:	Abstract:	Subject:	Abstract:
Antimicrobial Photodynamic Treatment	RM1	HACCP	RA-1
Bacteriophage therapy	RM-2	Hepatitis A viruses	DM-1, DM-2
Bean sprouts	DM-4	<i>Listeria monocytogenes</i>	DM-3
Bean sprout spent irrigation water	DM-4	Microarray	DM-1, DM-2
Cost-effective interventions	RA-1	Microfiltration	DM-4
Dephi survey	RA-1	Multiplex PCR	DM-1
DON	DM-3, RM-3	Mycotoxin	DM-3, RM-3
<i>E. coli</i> O157:H7	DM-4, RM-1	Noroviruses	DM-1, DM-2
Electrochemical Immuno-sensors	DM-4	Patulin	DM-4, RM-3
ELISA	DM-3	Pork	RM-2
Enteric viruses	DM-1, DM-2	Ready-to-eat foods	DM-2
Grains	DM-3, RM-3	RT-PCR	DM-1, DM-2
		<i>Salmonella</i>	RM-2