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# Innovation Express

Science and Technology News from Agriculture and Agri-Food Canada

## Addressing threats to the agricultural value chain

One of the major scientific challenges facing the world in the 21st century involves understanding and addressing threats throughout the agricultural and agri-food production systems. Pressures from weeds, insects, and diseases not only decrease crop yields, but in the case of quarantined pests, their presence can close our borders and impact our export markets. Pathogens such as virus, bacterium or other microorganisms that produce diseases can also impact the food processing supply chain – resulting in food recalls and illnesses.

Agriculture and Agri-Food Canada (AAFC) plays a key role in addressing these current and future threats. We work closely with colleagues throughout AAFC and the Canadian Food Inspection Agency (CFIA), providing scientific advice to guide our policy and regulatory decisions. Our partnership with the CFIA involves assisting in diagnosing new pests, especially invasive and quarantine species, and developing new diagnostic tools.

Recently our scientists not only revealed the presence of trunk diseases affecting vineyards in British Columbia, they developed a diagnostic tool so powerful it can simultaneously detect and identify all pathogens responsible for the decline in young vines. We have also developed a laboratory test that uses genetic markers (DNA barcoding) to quickly identify five species of cutworms – a type of caterpillar that feeds on canola, forage and cereal crops and has been of particular concern on the Canadian prairies in recent years. These teams continue to work with industry and our research partners on finding solutions to minimizing the impact these diseases and pests have on our production systems.

On the agri-food side, we have developed a method for quickly and reliably enumerating live *E. coli*, which can contaminate meat and cause toxic infection (food poisoning) in humans. Our scientists continue to be involved in projects that will improve pathogen detection, establish optimal decontamination strategies, and reduce food safety risks through alternative treatment and management strategies.

These are only but a few examples of how AAFC science helps protect our food supply, supports other government departments and ultimately benefits Canada's agriculture and agri-food industry. We hope you enjoy reading about these and other discoveries in this issue of Innovation Express.

Dr. Brian T. Gray, Assistant Deputy Minister and
Dr. Gilles Saindon, Associate Assistant Deputy Minister
Science and Technology Branch
Agriculture and Agri-Food Canada

## Inside



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## Nematology discoveries in Canada

Agriculture and Agri-Food Canada (AAFC) is at the forefront in combatting invasive and quarantined nematodes that threaten Canadian agriculture. Nematodes, simple and un-segmented microscopic roundworms or threadworms, are the most numerous multicellular microscopic animals on earth. They are one of the units of the National Biological Collections in Ottawa. Here AAFC scientists use the collection not only for taxonomic research, but also to differentiate and properly identify nematodes for researchers from federal and provincial institutions and universities across the country.

AAFC's Dr. Qing Yu is the curator of the Canadian National Collection of Nematodes located at the Eastern Cereal and Oilseed Research Centre (ECORC) in Ottawa, Ontario. His research is funded under the Genomics Research and Development Initiative (GRDI) and the Pulse Science Cluster *(Growing Forward 2)*.

**"Prompt and accurate identification of nematode species is vital to helping scientists, farmers and exporters respond to threats," says Dr. Yu.**

"We work closely with scientists from the Canadian Food Inspection Agency (CFIA) in developing new identification techniques and support two diagnostic laboratories at CFIA that are responsible for detecting nematode species."

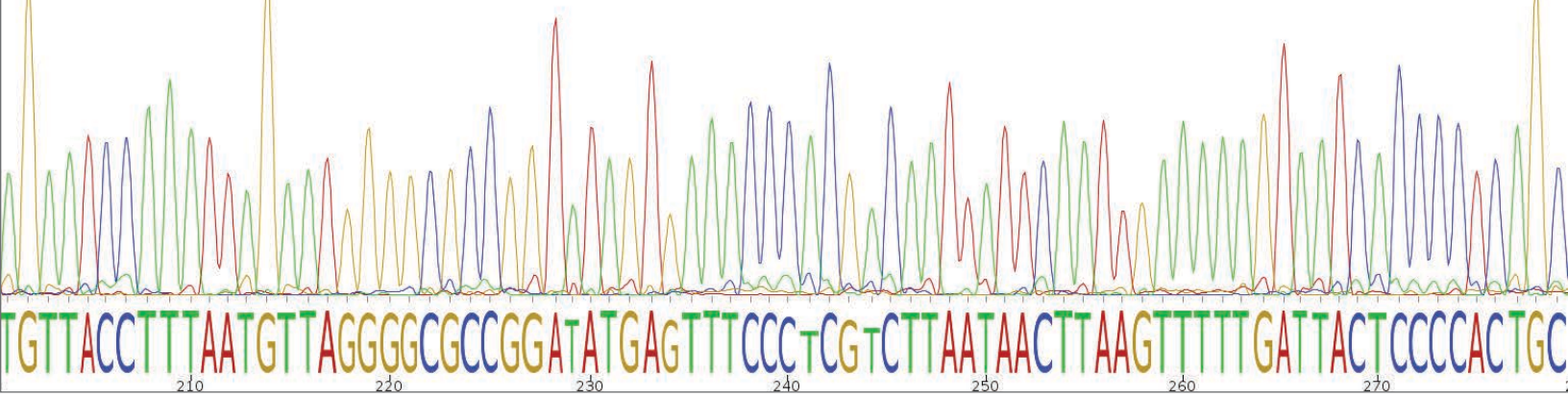
In 2013 alone, AAFC scientists used a combination of taxonomy and molecular tools such as DNA sequencing to discover and identify four invasive or quarantined nematodes: *Ditylenchus destructor/D.* dipsaci on garlic in Ontario; *D. dipsaci/D. weischeri* on peas in the Prairie Provinces; and the soybean cyst nematode *(Heterodera glycines)* on soybean in Quebec. These species pose serious threats to other crops as well. Following the discoveries, strict phytosanitary measures and recourse actions were implemented. Early detection and accurate identification saved Canadian farmers from potential serious losses.

### Did you know?

- The Canadian National Collection of Nematodes was created by A.D. Baker in 1943, and is the primary depository for Canadian nematodes excluding parasites of humans and other animals. There are nearly 30,000 slides and 5000 vials of over 88,000 roundworm specimens, including approximately 5000 primary and secondary types of nearly 400 species.

**The National Biological Collections comprise:**

- The Canadian National Collection of Insects, Arachnids and Nematodes (CNC), containing over 17 million specimens and one of the five largest collections of its kind in the world;
- The Glomeromycota in vitro Collection (GINCO) holding 94 mycorrhiza isolates on plant hosts;
- National Mycological Herbarium (DAOM) containing 350,000 specimens, including documentation of plant diseases and Canadian fungi spanning over 100 years;
- Canadian Collection of Fungal Cultures (CCFC) - a living fungal collection with over 17,000 cultures; and
- The National Vascular Plant Herbarium (DAO) - with 1.5 million specimens is one of the largest vascular plant collections in Canada.




## Next Generation Sequencing to identify pathogens

Agriculture and Agri-Food Canada (AAFC) scientists are playing a key role in accurately scanning, analyzing and documenting pests that have a detrimental impact on import and export commodities. Of particular concern are pathogens that can either cause significant yield loss or impose health risks to livestock and humans.

Dr. Wen Chen at AAFC's Eastern Cereal and Oilseed Research Centre in Ottawa, Ontario, uses Next Generation Sequencing (NGS) technologies to detect regulated pathogens at low levels. However, it takes a trained expert to avoid misinterpretation of NGS data.

Next Generation Sequencing is a tool used by scientists to extract genetic information from biological systems – plants, animals, bacteria, etc. The process figures out the sequence of millions of pieces of DNA all at once, enabling rapid sequencing of large stretches of DNA base pairs spanning entire genomes. It produces hundreds of gigabases of data in a single sequencing run.

"Most 'off the shelf' tools for automated classification and analysis of NGS are based on sequence similarity and are not useful in detecting very closely related species," says Dr. Chen. "For many of these species, when only sequencing information of targeted DNA regions are available, the accurate assessment of samples for the presence of a regulated pathogen or its close relative(s) requires additional or customized software and knowledge. As NGS technologies become readily available to many international users who may not have the required scientific expertise, misinterpretation of NGS data may generate 'false positive' results and create barriers to trade."

To prevent false positives and any potential trade issues, AAFC experts trained in taxonomy and genomics are developing bioinformatics tools to help scientists make the best and most accurate science-based decisions using NGS technologies. For example, tools are being developed to differentiate between pathogenic and non-pathogenic species amongst those that are closely related (available at Atlassian Bitbucket AODP_V2.0 release and Atlassian Bitbucket OFP as well as one that profiles the biodiversity of microbes and monitors pathogens present in commodities and different environments of the agri-ecosystem (available on CRAN) These tools are still under development for improved efficiency or expanded functions.

**AAFC scientists work in close collaboration with the Canadian Food Inspection Agency and the Canadian Grain Commission to help them accurately identify pathogens, support regulations for domestic products and export markets particularly for the grain sector. This collaboration helps protect Canada's trade efforts, and ultimately avoid new outbreaks and long distance dispersal of new invasive species.**

"Together we continue to develop the best science-based approach to accurately scan, analyze and document such economically important pests in import and export commodities to maintain our leadership role in protecting trade and reducing risks to the Canadian and North American agriculture industry," says Dr. Chen.

### Did you know?

Over 1200 fungal species are currently regulated (their presence monitored) by 15 countries / regions with 38 species in Canada and over 50 in the United States under quarantine surveillance.

## Partnership mitigates the re-emergence of Goss's disease in corn

In partnership with the Canadian Food Inspection Agency (CFIA) and the Manitoba Corn Growers Association (MCGA), Agriculture and Agri-Food Canada (AAFC) scientists have developed a new diagnostic tool to identify and help mitigate the re-emergence of Goss's disease in corn. Goss's disease, a bacterial disease that can cause up to 50% crop loss during severe epidemics, was first reported in 1969 in south central Nebraska, became sporadic after partially resistant corn cultivars were identified and cultivated but has recently re-emerged.

Dr. James Tambong at AAFC's Eastern Cereal and Oilseed Research Centre (ECORC) in Ottawa, Ontario used data generated from next generation sequencing technologies to develop a molecular multiplex diagnostic tool with high throughput capability for rapid and accurate detection of the pathogen in a small piece of infected corn leaf in less than five hours.

"Pathogens such as Goss's disease are constantly evolving new pathogenicity traits (ie .changing their genetic capacity to cause a disease) to enhance their virulence and adaptation to the hosts," says Dr. Tambong. "As with all diseases, early and reliable detection is the key to developing successful disease management strategies."

***This new diagnostic tool will be used during the 2015 corn-growing season to process infected corn leaf samples received from the MCGA.***

The genome-based tool is being validated by the CFIA for routine application in risk assessment of the pathogen for Canadian corn-growers. It will be employed for Phytosanitary Certification by the CFIA proactively or in compliance with the requirements of our trade partners, resulting in increased confidence in the Canadian agricultural system.

A Phytosanitary Certificate (issued by an exporting country) certifies that the plants or plant products covered by the certificate have been inspected according to appropriate procedures and are considered to be free from quarantine pests and practically free from other injurious pests, and that they are considered to conform to the current phytosanitary regulations of the importing country.

"Our science team is also studying the population structure of the bacteria collected between 1969 and 2010 to see if the DNA of recent strains is different," says Dr. Tambong. "New genotypes or variants of the strains identified in this study could be incorporated in future corn breeding programs to develop tolerant/resistant varieties."

This work is strengthening the competitiveness and sustainability of the Canadian corn industry and contributes to collaboration with the CFIA and industry to improve plant health management and other initiatives.

### Did you know?

The National Identification Services (NIS) at ECORC provide species identification and biological information services to identify pests, weed, fungi and bacteria impacting Canadian agriculture, in part to fulfill legal obligations required by various federal and provincial legislations (weed control, seed act, plant protection act, import and export rules, and customs regulations).

Thousands of authoritative identifications of quarantine and invasive plant species and weeds, invasive insect pests, nematodes, and fungal and bacterial diseases are provided every year supporting CFIA to prevent their spread or their entry into Canada and reducing and preventing economic losses to industry for exports.

## New on-line tools to identify stored grain insects

There is a zero tolerance for live insects in Canadian stored grain, which helps maintain Canada's reputation as a provider of high quality grain to customers around the world. Now, growers and elevator operators can identify stored grain insects using their computer or smart phone.

New on-line guides are the result of collaboration between Agriculture and Agri-Food Canada, (Drs. Yves Bousquet - Eastern Cereal and Oilseed Research Centre; Paul Fields and Noel White - Cereal Research Centre), the Canadian Grain Commission, University of Manitoba, and Soliton Apps. Together, they have developed two easy-to-use visual identification guides to allow non-experts to identify the most common stored grain insects. The guides use high resolution photos and simple non-technical language to help users identify insects.

***With over 100 insect pests that can occur in stored grain in Canada, identifying the correct insect is crucial to implementing the most effective control method.***

The guides cover the most common insects: 14 primary insect pests, and 20 secondary pests. The guides are available on the mobile friendly Canadian Grain Commission website (http://www.grainscanada.gc.ca/storage-entrepose/keys-cles/iik-cii-eng.htm), and as a stand-alone iPhone app called *StoredInsects*. Advice on sampling and control is also provided.

## Grapevine trunk diseases discovered in Canada

Grapevine trunk diseases are one of the biggest problems wine grape industries face around the world. With five different grapevine trunk diseases, each caused by many different fungal pathogens, this issue is a top concern for the wine industry. Researchers at Agriculture and Agri-Food Canada (AAFC) revealed for the first time that these diseases and pathogens occur in Canada and are helping the industry develop strategies to control them.

Dr. José Ramón Úrbez-Torres leads the research at AAFC's Pacific Agri-Food Research Centre (PARC) in Summerland, British Columbia with colleagues Dr. Pat Bowen and Biologist Dan O'Gorman. So far, the PARC team has identified over 30 pathogens that cause grapevine trunk diseases in British Columbia, and have entered this information into a DNA database.

"After studying the pathogens causing trunk diseases in British Columbia, we discovered several different fungal pathogens, including two new species," says Dr. Úrbez Torres. "The incidences of grapevine trunk diseases such as Petri disease and black foot, which cause a syndrome known as the young vine decline, have been rapidly increasing in California, Europe, and South Africa since the mid-1990s. We've now found that they are the most prevalent diseases in young vineyards in British Columbia as well."

***With over 80 different fungal species associated with grapevine trunk diseases worldwide, identification of these pathogens requires taxonomical skills and expertise in mycology.***

Furthermore, the current tools available for species discrimination are expensive and time consuming. On this front, the research team has also recently developed a molecular diagnostic tool so powerful it can simultaneously detect and identify all pathogens responsible for decline in young vines. They are working with the B.C. Ministry of Agriculture to provide training for a potential use of this molecular diagnostic tool at the B.C. Ministry's diagnostic laboratory, located in Abbotsford.

"We hope that eventually, growers could send in samples from vineyards or orchards, and quickly receive a DNA screening, identifying present pathogens and potential diseases," says Dr. Bowen. "Providing immediate diagnostic results and access to effective control and prevention methods will revolutionize the B.C. wine grape and tree fruit industries."

The natural next step in this research involves studying the pathogens and developing effective control mechanisms.

With funding through AAFC's *Growing Forward 2's* Agri-Innovation Program (awarded to the B.C. Wine Grape Council), the team is now examining specific vineyard conditions that promote infection – how they spread from plant to plant, how environmental conditions affect them, and how the diseases develop within the plants. Knowing that the pathogens primarily infect grapevines through pruning wounds, the researchers will be able to advise growers on when to prune their vines, and which biological and chemical controls and cultural practices will minimize the infection and spread of the diseases.

Ultimately, the PARC research team is seeking to develop effective chemical, biological, or environmental control strategies for different grapevine trunk diseases. These control methods will help growers in Canada, and across the globe, keep their vines healthy and productive.

### Did you know?

Canada's wine industry produces over 50 million litres of wine per year, worth more than $1 billion. The industry's economic value to Canada is nearly $7 billion per year, including tourism and taxes, according to a recent Frank, Rimerman and Company report.

**AAFC has developed several vineyard-related videos:**

Over Wintering Strategies: New vineyard management practices

Cover Crops: For the control of vineyard pests

You can also find them on our YouTube site: https://www.youtube.com/user/AgricultureCanadaEng

## Biocontrol agent *Hypena* against Dog-Strangling Vine

Biological control - the use of live organisms such as viruses, bacteria, fungi, insect parasitoids (which live on and kill their hosts) and predators (which attack and feed on other insects) to control insects, diseases and weeds - has a long history of success in Canada. It is often used to combat invasive species, helping to stop pests from reaching thresholds where farmers can experience economic loss. After years of research, this strategy is being used by Agriculture and Agri-Food Canada (AAFC) scientists to combat the dog-strangling vine (DSV), an invasive species believed to have been brought to North America from the Ukraine in the 1800s. DSV has now become a significant ecological threat to Canadian habitats, including rare species in Ontario's Carolinian forest region. DSV is considered a noxious weed in Ontario under the Ontario Weed Act and as such must be controlled before it becomes a major agricultural issue.

Dr. Rob Bourchier from the Lethbridge Research Centre in Alberta, and Dr. Peter Mason at the Eastern Cereal and Oilseed Research Centre (ECORC) in Ottawa are leading the project in collaboration with scientists from the University of Toronto, Carleton University, University of Rhode Island and the forest management company, SilvEcon Inc. DSV, which is also known as swallowwort, is from the milkweed family, and is of particular concern because it has been spreading rapidly with no known natural enemies on this continent. The weed forms dense mats that 'strangles' other vegetation, crowd out native plants, interfere with forest regeneration and interfere with agriculture production through invasion of field edges, planted crop fields and pastures where livestock forage.

The moth, *Hypena opulenta*, which like DSV is native to Ukraine, was first identified by researchers from University of Rhode Island and the Commonwealth Agricultural Bureau International (CABI) as a possible defense against the vine in 2006. Prior to its approval for release in 2013, the insect underwent extensive testing by the international team to

Image by A. Brauner

confirm that it can only survive on DSV and will not feed on native plants. The *Hypena* release program started in the fall of 2013 to test overwintering in the Ottawa area and was scaled up in 2014 with over 12,000 caterpillars released at Ontario field sites to date.

"Results from releases this summer (2014) have been very encouraging," says Dr. Bourchier.

***"Defoliation of DSV at release points is easily detectable and entire DSV plants are yellowing in response to insect feeding on just a few leaves. The Hypena caterpillars have already completed a full generation, from egg to adult and are into a second generation at Ottawa and Toronto release locations."***

The AAFC team will continue to monitor these initial release locations for additional impact on the DSV plants, spread of the insects and successful overwintering. These "nurse" sites will form the basis of a larger release program planned throughout Ontario in 2015. Studies are also underway to effectively integrate *Hypena opulenta* with other management tools being used for DSV.

"Once established at the initial release sites, *Hypena* moths will disperse to find new patches of DSV," says Dr. Bourchier. "*Hypena* caterpillars will defoliate the DSV and because they can only survive on DSV, their populations will rise and fall with the availability of their host plant. This long-term proposition will take several years to get caterpillar populations widely established and observe impacts over the large areas affected by dog-strangling vine."

There is no shortage of food out there as DSV has over a one hundred year head start on the caterpillars in Ontario. However, over the long term, the release of *Hypena* is expected to re-establish an ecological balance between the introduced weed and a natural enemy, which will help restore native habitats in Ontario and assist crop and wood producers.

## Did you know?

Dog-strangling vine can produce up to 28,000 seeds per square metre. The seeds are easily spread by the wind, and new plants can grow from root fragments, making it difficult to destroy. The vine has invaded ravines, hillsides, fence lines, stream banks, roadsides and utility corridors, and is also found in prairies, alvars (limestone plains), plantations of pine trees and natural forests.

AAFC's Canadian National Collection of Insects, Arachnids and Nematodes (CNC) is used for the development of mitigation strategies involving the discovery and use of appropriate biological agents to control pest insects. More than 10,000 insects belonging to 12 species were processed through the AAFC Containment Laboratories in 2009-2014 for lab studies or field release in four provinces against two insect pests and four weed species.

## Safer meats for Canadians

Ready-to-eat (RTE) meat products, such as deli meats, are being studied by Agriculture and Agri-Food Canada (AAFC) scientists to ensure their safety and find new ways to make healthier products. A team led by Dr. Sampathkumar Balamurugan of AAFC's Guelph Food Research Centre in Ontario, is working on two research studies to ensure that industry processing techniques of RTE meats provide the safest and healthiest food options for Canadians.

### Reducing harmful bacteria in sausage

In Canada, food safety guidelines for meat processing companies are established by Health Canada. Any deviation from these guidelines requires meat processing companies to scientifically validate processing methods by demonstrating they control pathogenic bacterial populations in their products. The most common way of achieving this is by heating the product to kill bacteria. However some traditional RTE meat products, such as salami, are not cooked but are fermented and dry cured.

Dr. Balamurugan is examining how different fermentation and dry curing processes impacts seven serotypes of *E. coli*, including O157:H7 and six non-O157, but still pathogenic, in dry-fermented sausages. *E. coli* O157:H7 is a serious disease-causing food-borne bacteria. Processing techniques used in industry must reduce *E. coli* O157:H7 as per regulations, but it is not clear if they will control other pathogenic serotypes of *E. coli*.

***"This research will lead to a better understanding of how the food industry can better prevent foodborne illnesses and how pathogens respond to food," says Dr. Balamurugan.***

"This research does not have to be limited to *E. coli*. Other common food-borne pathogens like Listeria monocytogenes and Salmonella could also be tested this way to ensure RTE meat food safety for Canadians."

### Impacts of salt reduction

Salt present in RTE meats serves a vital role as a food preservative to enhance shelf life and functionality in terms of flavour and palatability. However, today's consumers want healthier food options and this is frequently achieved by reducing salt content in food. Dr. Balamurugan is examining how reducing sodium chloride (salt) in RTE meats and replacing it with an alternative compound, like potassium chloride or calcium chloride, will impact shelf life in these products. He is specifically looking at RTE meats that have been processed using a high pressure/cold pasteurization technique, which kills all bacteria present with minimal changes to the characteristics of foods.

"We found that reducing salt content in RTE meats actually increases the efficiency of high pressure treatments," says Dr. Balamurugan. "Higher efficiency means reduced treatment times, which saves energy and reduces processing costs, which is beneficial for industry and the environment."

### Did you know?

These research projects were conducted at the Guelph Food Research Centre's Pilot Plant. The Pilot Plant is a unique food research facility that enables researchers to perform highly sophisticated and controlled experiments involving pathogens in a safe environment.

## Faster detection of *E. coli*

Agriculture and Agri-Food Canada (AAFC) scientists have developed a method for quickly and reliably counting the number of live *E. coli*, which can contaminate meat and cause toxic infection (food poisoning) in humans. This new method will be used by regulatory bodies and the meat industry to help them determine how much of the organism is initially present on meat and the success of decontamination techniques.

Dr. Xianqin Yang, at AAFC's Lacombe Research Centre in Alberta led the project team with funding by AAFC, the Beef Cattle Research Council, and the Alberta Livestock and Meat Agency.

"The industry is looking for quantitative methods with shorter turnover times to reduce the cost of testing and minimize the time products are held while being tested," says Dr. Yang. "With current methods, the biochemical technology used (real-time Polymerase Chain Reaction or PCR) detects the DNA from the bacteria, but it can't show whether it is DNA persisting in the environment or from bacteria, let alone differentiate live bacteria from dead ones. In the case of *E. coli* O157:H7, it is only the live bacteria that pose a risk to public health."

To control the contamination of beef with *E. coli* O157:H7, many large beef packing plants in North America have implemented decontamination techniques, particularly in the carcass dressing process. Those techniques include heat treatments, use of lactic acid and oxidizing agents that can kill bacteria.

"Since the available PCR-based methods could not differentiate DNA live bacteria from the dead ones, the efficacies of those treatments cannot be rapidly determined," says Dr. Yang.

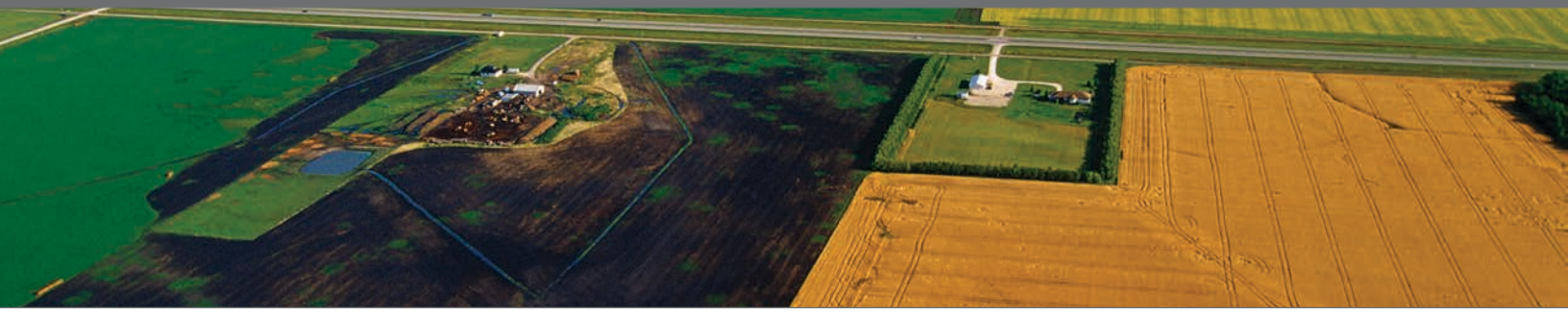
***"Thus, the ability to differentiate live and dead bacteria using rapid PCR methods allows researchers to detect whether there is a risk of contamination and the extent of contamination, and whether or not decontamination has been effective in killing the bacteria in real time."***

The new method for detecting *E. coli* developed by Dr. Yang's team tallies only live pathogens (real risk) by using real-time PCR in conjunction with sodium deoxycholate or sarkosyl, a type of biological detergent.

Dr. Yang is currently testing the robustness of the method at beef packing plants with the goal of safer meat and better decontamination techniques.

### Did you know?

- Regulatory authorities in North America require testing of carcasses, beef trimmings and ground beef for the presence of the noxious bacteria *E. coli* O157:H7, a subgroup of *E. coli* that can cause infections typically characterized by diarrhea and hemorrhagic colitis, and lead to hemolytic uremic syndrome (HUS), a life-threatening chronic condition with a 5% fatality rate.
- AAFC scientists also led a project to determine how beef carcass dressing and breaking processes, carcass and cut treatments and cleaning of both plant (for example, conveyors) and personal (knives and gloves) equipment affect the microbiological condition of beef carcasses, cuts and trim. (See: Evaluation of Beef Carcass Processing Procedures, Innovation Express, Vol 5, No 3).



## Tell Us What You Think

*Innovation Express* is Agriculture and Agri-Food Canada's quarterly news magazine to promote research partnerships and technology transfer to organizations interested in agri-food research and development.

**We welcome your comments and suggestions.**

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