The 2019 AAFC-USDA meeting on genotyping and monitoring of high-risk plant pathogens

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Abstract

Chief scientists from USDA and AAFC both agree that genotyping and monitoring of high-risk plant pathogens remains one of the five strategic priority areas for focused collaboration. Research scientists from both organizations met on August 3, 2019 in Cleveland, Ohio to update an Action Plan from 2014. Many aspects around genotyping and monitoring of high-risk plant pathogens were discussed. It was agreed that high risk pathogens have certain common characteristics such as being seed-borne, soil-borne, hard to manage once established, and a potential major economic impact. Some specific high-risk pathogens proposed to study were carried over from the 2014 Action Plan. The researchers emphasized the need for better communication and resource sharing. A management driven project from both the USDA and AAFC would allow this team and network to really develop a collaborative effort with innovative results. In order to achieve its full potential, full support by the Chief Scientists from USDA and AAFC with dedicated research funds will be needed.

Introduction

The USDA Chief Scientist at the time, Dr. Catherine Woteki, and the AAFC Assistant Deputy Minister of the Science and Technology Branch at the time, Dr. Siddika Mithani, met in Ottawa at the AAFC headquarters in May 2013. Because resources are always scarce, they agreed it would be beneficial for both organizations to collaborate and really focus on five strategic priority areas (File S1). One of those strategic priority areas was identified as next-generation sequencing technologies and applications for genotyping and monitoring of high-risk plant pathogens. Coordination would be led by designated points of contact who would communicate with each other to explore collaboration opportunities and take action as appropriate. This led to a two-day meeting held in June 2014 in Beltsville, Maryland where a group of scientists from USDA-ARS, USDA-APHIS, AAFC and CFIA met. They discussed six major topics around fungal and bacterial pathogens (File S2) that included: 1) Defining high risk plant pathogens and prioritizing them; 2) Acquiring, sharing, and analyzing big data, including hardware, software, and approaches to bioinformatics analysis; 3)

Sequencing whole genomes of selected pathogens; 4) Detection, identification, building reference data sets, methods of analysis, air, rain, commodity sampling for monitoring pathogen distribution & spread by metagenomics approaches; 5) Distinguishing dead from alive with molecular detection methods; and 6) Updating taxonomic monographs for important plant pathogenic groups or genera. They drafted an Action Plan that was set for 3 to 5 years (File S2). Some of the original items from the 2014 Action Plan were successfully completed while others were missed due to changes in staff or lack of funding.

On July 20, 2018, the Acting USDA Chief Scientist, Dr. Chavonda Jacobs-Young, wrote a letter to AAFC's Assistant Deputy Minister of the Science and Technology Branch, Dr. Brian Gray (File S3) expressing that monitoring and genotyping of high-risk plant pathogens remains one of the five priority areas for collaboration going forward. Dr. Gray responded back with a letter acknowledging and agreeing to the contents of Dr. Jacobs-Young's letter (File S4). On October 29, 2018, Dr. Jacobs-Young, wrote a second letter to Dr. Gray (File S5) expressing that it is necessary to meet and discuss development of a new Action Plan on genotyping and monitoring of high-risk plant pathogens that would incorporate updated research priorities, as the 2014 Action Plan is almost 5 years old. Dr. Jacobs-Young stated in her letter that "The main goal of the new Action Plan will be to speed up the creation and adoption of technological innovation that use advancements in sequencing technology, bioinformatics, and artificial intelligence to prevent the introduction and spread of high-risk plant pathogens into Canada and the United States."

Dr. Tim Widmer, USDA-ARS National Program Leader for Plant Health, and Dr. Hai Nguyen, AAFC Research Scientist, organized a satellite meeting on August 3, 2019 at the 2019 American Phytopathological Society (APS) meeting in Cleveland, Ohio where a group of scientists (Figure 1) from both organizations met to discuss a new Action Plan. A website hosted by USDA/ARS was set up to document the event (http://www.ars.usda.gov/research/aafcusdacollaboration). The previous Action Plan focused mostly on bacterial and fungal pathogens and was tied to the AAFC project, "Next generation sequencing – genomics and metagenomics of quarantine fungal and bacterial crop

pathogens," which concluded in March 2018. The new Action Plan was expanded to also include oomycetes, nematodes, viruses and phytoplasmas because some species in these groups of organisms are considered high-risk plant pathogens. In attendance at the meeting were seven USDA-ARS, one CFIA, and ten AAFC researchers who gave 10-minute presentations on their area of expertise and research. The presentations are posted on the event website. Five USDA-ARS, one USDA-APHIS, and two AAFC researchers were not able to attend but their interests were represented by their colleagues.

Oral presentations by the attendees were mainly focused on the scientists' specific research program and area of expertise in order to be informative and form closer collaborations. The presenters acknowledged some of the existing collaborations but also proposed potential areas for new collaborations between scientists or their counterparts on each side of the border. On a broad level, this included: 1) resource sharing of herbarium material, data or next generation sequencing protocols; 2) knowledge of taxonomy & nomenclature of specific species of concern; 3) comparative genomics that is focused on the study of gene clusters and toxin production; and 4) standardization of methods and data.

During the coffee breaks, lunch break and group discussions, participants had a chance to meet with their counterparts and discuss common areas for collaboration on high risk plant pathogens. Towards the end of the day, two discussion groups were formed to help the drafting of a new action plan.

There was a consensus that high risk pathogens have certain characteristics such as being seed borne, soil borne, hard to manage once established, and would have a major economic impact. Both the USDA and AAFC/CFIA are not well prepared to develop diagnostic methods as the research is often reactive rather than predictive. Some specific high-risk pathogens proposed to study for this Action Plan were carried over from the 2014 Action Plan. The group will keep an updated list of pathogens important to work on while taking into consideration the expertise and interest of the new research members. The researchers emphasized the need for better communication and

resources sharing, such as having access to the holdings of the biological collections in Ottawa, Beltsville, or the holdings of their own research labs. The participants recognized and suggested that collaboration on existing projects, pre-dating this new Action Plan, would be a convenient and cost-effective approach to move forward, but real collaboration would remain weak. A more sustained or incentivized approach would be to have a coordinated management driven project from both USDA and AAFC sides, with dedicated research funds, that would allow this team and network to really develop. A dedicated project forces the team to plan out their goals together and holds the team accountable to achieving objectives and milestones while making collaboration between USDA-AAFC on genotyping and monitoring of high-risk pathogens a tangible reality. Funding dedicated to this important collaborative effort will need to be initiated by decisions of the Chief Scientists from USDA and AAFC.

Figure 1. Photograph of some of the participants taken at the meeting closure at the Huntington Convention Centre in Cleveland, Ohio, United States. Top row from left to right: Oualid Ellouz, Mamadou L. Fall, Linda Harris, Sarah Hambleton, James Tambong, Niklaus Grunwald, Elizabeth Rogers, Jo Anne Crouch, Miao Liu, Kai Ling, Jonathan Griffiths. Bottom row from left to right: Jeremy Dettman, Timothy Widmer, Hai Nguyen, Wen Chen, Tahera Sultana, Sean Li, Walter Mahaffee

With input from the scientists, a draft Action Plan is presented below. Sometimes, researchers are already loosely working together because their independent projects, either from AAFC or USDA-ARS, are similar in nature. However, this Action Plan is only a draft because it is for the most part, non-committal, unless there is an overarching funded project with objectives and milestones binding scientists USDA-ARS and AAFC and holding them accountable.

The 2019 Action Plan (DRAFT)

1. Collaboration on specific high-risk pathogens
    a. Fungi
        i. Identification of closest neighbor to *Synchytrium endobioticum* (Nguyen/Bienapfl)
        ii. Powdery mildews (Liu/Crouch)
        iii. *Tilletia* (Hambleton/Salgado-Salazar)
        iv. Rust (Hambleton/Castlebury)
        v. *Fusarium* species associated with Fusarium Head Blight - sharing knowledge and developing tools to track the mycotoxigenic potential of FTSC (*Fusarium tricinctum* species complex) FTSC and FSAMSC (*Fusarium sambucinum* species complex) populations, specifically targeting known and novel mycotoxins which threaten the safety of food and feed chains (Harris & Overy/Kistler & Cowger)
    b. Bacteria
        i. Generating reference genomes for *Xyllela fastidiosa*, *Ralstonia*, *Clavibacter*, *Dickeya* (Tambong/Rogers)
    c. Oomycetes
        i. Characterization of downy mildews using target enrichment (Nguyen, Dettman & Hambleton/Crouch)
        ii. Oomycetes detection with metabarcoding and whole genome sequencing with focus on *Pythium* & *Phytophthora* spp. (Nguyen & Chen / Martin, Grunwald & Abad)
    d. Nematodes
        i. Molecular identification and whole genome sequencing of high-risk nematode species and their close relatives (Sultana/Carta & Skantar)

ii. Building an online database of high-risk nematode species (Sultana/Carta & Skantar)

e. Viruses and phytoplasma

i. Disinfection & monitoring of tomato brown rugose fruit virus (ToBRFV) (Griffiths & Fall/Ling)

ii. Grapevine viruses (Fall & Griffiths/Ling)

2. Updating the list of pathogens of concern for USA and Canada (Nguyen/Widmer)
3. Technologies
    a. Comparative analysis to improve the two bioinformatics pipelines (VirTool and VirusDetect) for efficient virus identification (Fall & Griffiths/Ling)
    b. Detection of pathogens and molecular epidemiology with bioinformatics pipelines and next generation sequencing technologies (NGS) (Chen/Grunwald)
4. Resource sharing
    a. Easier method of sharing specimens from the living culture collections between the USDA-ARS and AAFC through development of a generalized Material Transfer Agreement (MTA)
    b. AAFC collections need an online database that is searchable or an agreement that both AAFC and USDA to freely share data on the holdings between each other
    c. Improved communication between Canada and USA on genotyping and monitoring of high-risk pathogens to avoid repetition & redundancy of research (Nguyen/Widmer)
    d. Adaptation of the USDA bacterial inventory database system to collect bacterial pathogen cultures in Canada (Tambong/Rogers)

Guidelines and Principles for USDA-AAFC Joint Priority Science and Technology Research Areas (updated August 2018)

Since 2013, Agriculture and Agri-Food Canada (AAFC) and the U.S. Department of Agriculture (USDA) have had in place a voluntary cooperative framework for priority collaborative science and technology research (see Background). As identified priority research areas continue to change over time, with this document, both organizations have reassessed and further developed this original framework. This document does not create any legally binding obligations for participants.

Context: AAFC and the USDA have extensive science cooperation. Not only do Canada and the U.S. share ecosystems and have similar agricultural production systems, but many AAFC and USDA scientists have obtained university degrees and have professional contacts in both countries. A wide array of science cooperation between AAFC and USDA scientists occurs naturally. There are numerous agricultural research and development collaborations and partnerships occurring between AAFC- and USDA-funded scientists, and continued collaborations of this kind are encouraged.

Purpose: Since 2013, Chief Scientists from AAFC (Assistant Deputy Minister – Science and Technology Branch) and USDA (Under Secretary for Research, Education, and Economics), have met approximately twice per year to identify priority areas for cooperation to focus and strengthen the impact of bilateral cooperation. By exchanging information, linking networks and capturing synergies, both Canada and the U.S. benefit from each other's scientific capacities and generate more and better research results to support their respective agriculture sectors.

Priority Area Identification: Joint USDA-AAFC priority science and technology research areas ("priority areas") are identified and mutually agreed upon by the Chief Scientists of AAFC and USDA to make best use of scarce resources and to focus and strengthen the impact of bilateral research collaborations between the two organizations.

Significance: Priority areas are identified in order to encourage and focus attention on joint work for a period of time and for a shared purpose. For example, designation as a priority area may facilitate the development of needed connections between relevant researchers in the U.S. and Canada; or it may motivate the development of clear and achievable joint research goals and metrics to address shared challenges.

Objectives: Priority areas should have specific and measurable goals. If these goals change over time, this should be documented in the progress report (see Reporting).

Number: The Chief Scientists endeavor to focus on only those areas of highest priority that can benefit from increased attention to further their successes. Limiting prioritization to five or fewer areas may allow for substantive attention from the Chief Scientists. "Subthemes" should be included sparingly to avoid the dilution of focus.

Reporting: Progress reports have historically been developed and submitted as an appendix to the U.S.-Canada Consultative Committee on Agriculture (CCA), approximately every six months. In the absence or infrequence of CCA meetings, progress reports should be prepared no less than once per year. Reports should be concise and non-technical summaries of research progress and significant issues. Initial drafting typically alternates between the two countries, from one report to the next, as confirmed through discussions between the report drafters. Each priority area should have one or more scientific "Lead(s)" from each country. For each priority area, Leads from the two countries consult and provide a written update on progress towards joint research goals to the report drafters, who prepare the summary report. Reports are finalized following review by the Chief Scientists.

Graduation and Sunsetting: Priority areas are not intended to continue indefinitely within the priority area framework. The goal is to "graduate" the work for continuation outside of the framework once near-term objectives of prioritization are met, or to "sunset" the work with recognition that the goals have been achieved. Before the finalization of each joint report, the Chief Scientists will discuss candidates for graduation and sunsetting, along with potential new priority areas. "Graduation" should not be interpreted as the signaling of decreased interest or prioritization of the topic within either department, but rather a recognition that joint prioritization is no longer needed to achieve goals.

Background: At a meeting held on May 6, 2013 at AAFC headquarters in Ottawa, Canada, Dr. Catherine Woteki (Chief Scientist, USDA) and Dr. Siddika Mithani (Assistant Deputy Minister, Science and Technology Branch, AAFC) decided to work together to make the best use of scarce resources and focus on advancing collaboration around five strategic priority areas. A mutual framework, "to make best use of scarce resources and focus on advancing collaborations…," was memorialized in a letter from Dr. Woteki to Dr. Mithani, dated August 12, 2013. The following guidelines and principles were elaborated:

- There are over 100 known agricultural research and development collaborations and partnerships already occurring between AAFC and USDA funded scientists, and continued collaborations of this kind are encouraged.
- Additionally, AAFC and USDA will engage in frequent bi-lateral discussions, communications and exchanges as appropriate, with regard to engagement in multi-lateral fora.
- Pursuing enhanced collaboration in the five strategic priority areas listed above should not be interpreted to stifle, curtail, cease or otherwise deter ongoing collaborative efforts between the two countries on agricultural research and development.
- Designated lead points of contact as listed above for each strategic priority area will communicate with each other to explore collaboration opportunities and take action as appropriate over the next six months, to advance the science, cooperation and strategic planning in those areas listed.
- Implementation of any activities are subject to the availability of personnel, resources, and appropriate funds.
- A report out on progress made in the strategic collaboration priority areas outlined above will be made to the CCA in fall of 2013.

Since that time, USDA and AAFC have prepared joint reports, to coincide with the U.S.-Canada Consultative Committee on Agriculture (CCA), or at least annually in the absence of CCA meetings.

During their June 2018 discussion of USDA-AAFC priority cooperation, Dr. Brian Gray (AAFC) and Dr. Chavonda Jacobs-Young (USDA) discussed the potential value of developing revised guidelines and principles for joint science and technology priority research areas.

U.S.-Canada AAFC-USDA Cooperation in Agricultural Research and Development Meeting in Beltsville, Maryland June 2-3, 2014

Background: An agreement between Under Secretary Catherine Woteki and Dr. Siddika Mithani, Assistant Deputy Minister, Agriculture and Agri-Food (AAFC) Canada identified "Next Generation Sequencing Technologies and Applications" for genotyping and monitoring or quarantine and invasive species as a strategic priority area. This resulted in a meeting held at ARS, Beltsville on June 2-3, which included scientists from the Science & Technology Branch (STB) of AAFC, and from the Canadian Food Inspection Agency (CFIA), along with scientists from USDA's Agricultural Research Service (ARS) and the Animal, Plant Health Inspection Service (APHIS). Scientists drafted an Action Plan for collaborative research on next generation sequencing for plant pathogens of high consequence. This research will provide faster, more accurate diagnostic methods for these pathogens, and allow better monitoring of plant pathogen spread. This will facilitate trade in agricultural products and allow better predictive models for plant disease forecasting.

Brief report: Topics at this meeting were limited to fungal and bacterial pathogens (see agenda in Appendix I). Viruses, nematodes, and other pathogens will be discussed in the future. Most meeting participants had expertise in fungi or bacteria, and a few participants had expertise in bioinformatics and computer science. At the meeting, scientists identified collaborative projects already existing between Canada and the US on next generation sequencing, and sought ways to strengthen these partnerships. Scientists also discussed areas of need for new partnerships. Work falls into the categories of 1) defining high risk plant pathogens, 2) bioinformatics, 3) genomics, 4) metagenomics, 5) molecular detection (including distinguishing dead vs. alive), and 6) nomenclature and taxonomy.

1. Scientists were in general agreement on high priority pathogens for sequencing, but felt that additional information on how pathogens are prioritized by their respective regulatory agencies would be helpful. Thus, one action item is to obtain prioritized lists of pathogens from the regulatory agencies for future discussion.

2. The area of bioinformatics generated discussion in acquiring, sharing, and analyzing big data, including hardware, software (including training), and approaches to analysis. Action items include setting up a meeting between Canadian and US computer/bioinformatics/information technology specialists to determine how best to facilitate the mechanics of sharing big data between the two governments. A subcommittee has been tasked with collecting information on currently available large databases to find out what categories/parameters of data are collected for metadata purposes. This information will be used to define what categories of data we include as we build our databases and what we include in metadata associated with large raw datasets. Another concern was the curation of these databases and how changes in nomenclature and taxonomy updates could be captured so that all databases are harmonized for searches and comparisons. Development of tools for data analysis was considered a priority. Canada and the US will each hold a webinar for training in data analysis software they have developed and found useful. Canada will train in the use of AODP-OFP and the US will train on the use of EDNA.

3. Many of the existing and proposed collaborations are in the area of genomics. Collaborative projects in progress include graminicolous downy mildews, *Synchytrium endobioticum*, potato

zebra chip, and stem rust of wheat (Ug99). Collaborations for sequencing additional pathogens have been identified or are in the process of being identified and these are listed in the action plan.

4. Discussions on metagenomics centered on molecular detection and identification, building reference data sets for identification by sequencing PCR amplicons, and methods for analyzing data. Also discussed were air, rain, and commodity sampling metagenomics for monitoring pathogen distribution and spread.

5. Under the topic of molecular detection, there was discussion of distinguishing live propagules from dead. Current methods for making this distinction are not considered satisfactory. The possibility of partnering with scientists working on the Phytobiome and Soil Biome projects will be explored.

6. While nomenclature and taxonomy were not included as such on the original meeting agenda, these topics were discussed frequently. Nomenclature is the system of scientific names following international codes whereas taxonomy is the science behind defining species. International reference collections link both together by having the type specimens for described species. Identifications are rapidly becoming more precise due to molecular taxonomy tools, including sequencing entire genome to determine species boundaries and resolve complexes. For plant pathogens, it is critical to assign the correct name to the specimen in question since organisms with similar, if not identical, morphology can have very different biological characteristics including their ability to cause disease. Thus, some of the collaborations already in progress and those proposed are focused on taxonomic monographs and updating nomenclature in databases. Because of recent major changes in the *International Code of Nomenclature for algae, fungi, and plants*, a large proportion of fungal names will be changed, having a major impact on collection databases and metagenomics analysis tools.

Short term Action Items

<u>Within 30 days</u>
Draft brief report summarizing the meeting; distribute report to meeting attendees and solicit edits
Identify people to participate in action items in cases not identified during meeting
Post recorded meeting and slide sets online and provide link to meeting attendees
Set date for training on EDNA (metagenomics pipeline) - *will be 22 October 2014*
Set date for training on AODP-OFP (metagenomics pipeline) - *will be 30 October 2014*

<u>Within 60 days</u>
Finalize brief draft report from meeting and distribute to meeting attendees and others
Define the questions that will become first test cases for sharing Big Data and analysis tools (*will be* Tilletia *for fungi and* Rathayibacter *for bacteria, see Appendix II*)

<u>Within 90 days</u>
Hold meeting of collaborators in conjunction with the joint APS/CPS meeting August 9 – 13. Update on progress of collaboration and add time targets to Action Plan.

Action plan (3-5 years)

note: country origin of participants in this order (Canada/US)

1. Follow-up meeting between U.S. and Canadian computer/IT people
    a. Identify key players on each side (Lewis / Doreen Ware)
    b. Exchange documents/reports to help move forward on each side of border
2. List of pathogens of concerns (Levesque & Sean Li / Luster & Abad)
    a. Consolidate tiered lists, and revisit periodically
    b. Get information on level of risk
    c. Look at mechanisms to share this data
    d. Work with Mexico
3. Reference data for identification, nomenclature and pathogen information
    a. List of biological collections (Levesque & Redhead/ Schneider & Castlebury)
        i. Compile lists of biological collections at AAFC and USDA-ARS containing plant pathogens
    b. Coordinating database upgrades in AAFC and USDA for fungal collections (Redhead & Lévesque & Lewis / Castlebury)
        i. Compare database systems
        ii. Identify common fields
        iii. Evaluate possibility of harmonizing the two systems
    c. Link Canadian Host Pest Database and US Host Pest Database (Redhead & Lewis & Macklin / Castlebury)
    d. Giving the right names to priority lists, specimens, sequences, etc
    e.g. Single name search that includes all synonyms
        i. Nomenclature automatic updates of <u>fungi</u>
        (Redhead & Levesque & Lewis / Castlebury)
            1. Identify key players
            2. Harvest Index Fungorum and/or Mycobank
            3. Automatic updates from US and Canada to other databases (e.g. GenBank, Mycobank, etc)
            4. Use it in OTU assignment in taxonomic analysis pipelines
        ii. Nomenclature updating of bacteria)
        (Sean Li & Tambong / Carolee Bull)
            1. Establish mechanism
    e. Data curation of plant pathogen sequences for NGS data analysis (Levesque et al. / Castlebury / Bonants for Qbank in Europe)
        i. Look at UNITE and at Canada/US participation
        ii. Set up a meeting or conference call with UNITE with Canada/US participation (Lévesque)
4. Metadata for both genomics and metagenomics (Wen & Newton / Doreen Ware & Schneider)
    a. Exchange standards about metadata (Wen will send for genomics and metagenomics)
    (check Barcode of Life model for data release at Guelph, (Lévesque/Seifert)

    b. Subgroup to develop SOP for inclusion, release, etc
    c. Sharing current metadata
5. Raw data (Kandalaft / Doreen Ware)
    a. Sharing Big data
    b. Create technique for pre-screening pathogens of concern
    c. Stepwise release and validation before public release
    d. Build up redundancy between Canada and US before release
6. Bioinformatics tool development (Kandalaft & Lewis et al. / Doreen Ware)
    a. List of tools in Canada and US (e.g. Galaxy)
        i. Web Demonstration to US (Kandalaft)
        ii. Input necessary for tool
        iii. Output
    b. Eprobe and oligo pipeline
        i. Canada – US web demonstrations (Chen / Schneider)
7. Sharing molecular and robotics protocols (Levesque / Crouch)
    a. Gathering data on capability of different platforms
    b. Gathering protocols
8. Collaboration of specific pathogens
    a. Closest neighbor to Synchytrium endobioticum (Levesque / Castlebury)
    b. Phytoplasma collaboration (Crystel Olivier & Tim Dumonceaux & Sean Li / Bob Davis)
    c. Phytophthora typification (Levesque / Abad)
    d. Phytophthora detection (Lévesque & Bilodeau / Frank Martin)
    e. Pantoea (beneficial and pathogens) (Clavibacter) (Tambong / Loper & Wechter)
    f. Fusarium monograph (Seifert / O'Donnell & Geiser @ Penn State)
    g. Pythium monograph (Levesque / Abad)
    h. Rain and air sampling (unified system TBD)
    i. Mycotoxin producing Penicillia (Seifert / Peterson & Palumbo)
    j. Calonectria (Redhead & Tom Xsiang @ Guelph / JoAnne Crouch)
    k. Downy mildews in general (Levesque & Hambleton / Crouch)
    l. Magnaporthe – wheat blast (to be hired / Gary Peterson & Kerry Pedley)
    m. Ralstonia (Sean Li & James Tambong/ B. Schneider & Qi Huang)
    n. Tilletia controversa, indica and other species/ (Hambleton / Luster, Gary Peterson & Castlebury)
    o. Rust taxonomy (Hambleton / Castlebury)
    p. Wheat rusts (Hambleton / Szabo)
    q. Urocystis agropyri (samples) (Hambleton / Castlebury)
    r. Rathayibacter toxicus (Sean Li & Tambong / Schneider)

9. Dead vs alive detection
    a. Soil (Donna Smith / Bob Davis & Schneider)
    b. Spore collector (Levesque & Wen Chen / Schneider)

Appendix I: Agenda circulated for the Beltsville meeting

(edited to reflect some changes in schedule and attendance)

USDA-ARS and AAFC-STB Meeting

June 2-3, 2014

Next Generation Sequencing (NGS)
applying NGS towards monitoring and genotyping of high risk plant pathogens.

Location:
USDA-ARS George Washington Carver Center, 5601 Sunnyside Avenue
Beltsville, Maryland 20705
Room 4-2223

Web conference connection (detailed instructions attached)

1. Participant application - windows P.C.
https://connect16.uc.att.com/usda/meet/?ExEventID=86042415
2. Web browser application - Any Computer Type
Web Conference: https://www.connectmeeting.att.com
Meeting Number: 8888449904 - Meeting Code: 6042415

Participants:

USDA-ARS:

on site: Deb Fravel, Kay Simmons (day 2), Doug Luster, William Schneider, Lisa Castlebury, Jo Anne Crouch, Robert Davis, Glenn Hanes, Jack Okamuro (day 2),

AAFC-STB:

on site: André Lévesque, Wen Chen and Sarah Hambleton
via web: Denis Petitclerc (day 1), Michèle Marcotte, Chris Lewis, Keith Seifert, Iyad Kandalaft, James Tambong,

USDA-APHIS:

on site: Gloria Abad

CFIA:

via web: Guillaume Bilodeau (Ottawa, day 1), Sean Li & Donna Smith (Charlottetown)

Background: At a meeting held on May 6, 2013 at AAFC headquarters in Ottawa, Canada, Dr. Catherine E. Woteki, Under Secretary and Chief Scientist, U.S. Department of Agriculture and Dr. Siddika Mithani, Assistant Deputy Minister, Science and Technology Branch, Agriculture and Agri-Food Canada, decided to work together to make best use of scarce resources and focus on advancing collaboration around five strategic priority areas which included “Next Generation Sequencing Technologies and Applications”.

Summary of priority area and purpose of meeting: The price of DNA sequencing continues to decrease at a very fast rate. This offers both opportunities and some potential challenges. It is becoming affordable for almost any country to screen import commodities for pathogens

using metagenomics with next generation sequencing tools. In order to exploit properly the full capability of current and upcoming next generation sequencing for monitoring and genotyping, it is essential to have extensive reference sequence databases of all high risk pathogens and their close relatives for at least the most commonly used genetic markers (e.g. Internal Transcribed Spacer). It is also important to have complete genomes of all the high risk pathogens and their most virulent genotypes as well as genomes of some of their closest relatives whenever possible. The purpose of this meeting is to

- Identify and select collaborative projects in genomics of high risks pathogens.
- Identify and select collaborative projects in metagenomics (NGS sequencing of amplified genetic markers or shotgun sequencing)
- Identify gaps in reference sequence data for both full genomes and selected genetic markers such as rDNA or ITS and target AAFC and USDA collection accessions to be sequenced.

Agenda

Note: it is critical to respect the time allotted.

The goal is to get an overview of the work being done to see potential interactions/collaborations and not so much in getting the scientific details

Monday, June 2nd

9:00*	Introduction	Deb Fravel, USDA Denis Petitclerc, AAFC
	Review of driving forces for this meeting	
9:15	Summary of discussions of May 6th, 2013, and follow up at AAFC and USDA-ARS	André Lévesque, Doug Luster and Deb Fravel
	Defining High Risks Plant Pathogens	
9:45	Current scope of collaboration now and potential for expansion in the future	Doug Luster, André Lévesque, Deb Fravel, all
10:00	**Break**	
	Bioinformatics	
10:20	Bioinformatics and reference databases in Systematic Mycology and Microbiology	Lisa Castlebury, USDA-ARS
10:40	Bioinformatics platform and databases for AAFC Biodiversity, Shared Service Canada	Chris Lewis, AAFC
	Genomics – Bacteria/Phytoplasma	
11:00	*Rathayibacter toxicus* genome sequencing	Bill Schneider, USDA-ARS

* all times listed are Eastern Daylight Time

11:15	Mycoplasma, Phytoplasma, and Spiroplasma: Models for Synthetic Biology, and Invasive Plant Pathogens of Concern to North America	Bob Davis, USDA-ARS
11:30	Comparative genomics of Pantoea stewartii subspecies	James Tambong, AAFC
11:45	Genomics-based approaches for detection and identification of R. solanacearum race 3 bv 2	Sean Li, CFIA
12:00	**Lunch (at cafeteria)**	
	Genomics - Fungi	
1:00	*Tilletia* species	Sarah Hambleton, AAFC
1:15	*Synchytrium endobioticum* genome and genotyping	André Lévesque, AAFC, Guillaume Bilodeau, CFIA
1:30	*Penicillium verrucosum* (ochratoxin)	Keith Seifert, AAFC
1:45	Brief overview of some of the other high risk pathogen genomics projects at AAFC	André Lévesque, AAFC
2:00	NGS approaches to study emergent pathogens of ornamentals & turf	JoAnne Crouch, USDA-ARS
2:30	**Break**	
	Metagenomics	
3:00	Status of the molecular detection and identification of Graminicolous Downy Mildews of concern	Gloria Abad, USDA-APHIS
3:15	Building reference data for identification by sequencing of PCR amplicons	Sarah Hambleton, AAFC
3:30	Air, rain and commodity metagenomics with ITS amplicons	Wen Chen, AAFC
3:45	EDNA: E-probe Diagnostic Nucleic Acid Analysis for plant pathogens	Bill Schneider, USDA-ARS
	Molecular Detection (dead vs alive)	
4:00	*Synchytrium endobioticum*	Donna Smith, CFIA
4:15	General discussion on the issue	All
4:30	Summary of Day 1	
5:00	**Adjourn Day 1**	

Dinner in Beltsville (Luster, Schneider, Lévesque, Chen, Hambleton)

Tuesday, June 3rd

9:00	Introductory remarks	Kay Simmons, USDA-ARS Michèle Marcotte, AAFC
	Open data policy in US	Jack Okamuro, USDA-ARS
	Open data policy in Canada	Darren Cook, AAFC (presented by A. Lévesque)
10:00	**Break**	
10:30	Identifying areas for collaboration and complementarity as well as teams for each area	
12:00	**Lunch**	
1:00	Review of action items	
2:00	Adjourn meeting	
2:30	Visit of Mycology Herbarium	
5:00	Departure of AAFC participants to airport	

Appendix II: Two pilot projects for sharing big data through iPlant and KBASE

Test cases to use iPlant for fungal genomics for the USDA-AAFC collaborations

It has been agreed that iPlant would be the best platform to use for collaborative work on genomics of high risk fungi as it is more mature than K-base and is focussed on eukaryotes. We propose to work comparative genomics of *Tilletia* species as the first test case. AAFC has done five *Tilletia* species with Illumina sequencing, most with various insert lengths and RNAseq under various conditions for gene discovery. Assemblies were done at AAFC and genome annotation is underway. Because of the good quality of most of the data and because several analyses are already done on local server, it provides a good first test case to work collaboratively with iPlant. The goal is to obtain through comparative genomics robust markers to separate species readily. Since some species are very closely related and cannot be resolved with the standard taxonomy markers, it is very likely that the species need to be separated on the basis of genes related to host specificity (e.g. effectors). The following steps will be performed: assemble and annotate genomes, perform various comparative analyses of genomes (e.g. syntheny, identify genes unique to species), identify and compare the secretomes (e.g. effectors), identity a suite of unique markers for different *Tilletia* species. After the successful initial phases of test case with Tilletia specie, we would like to follow up with downy mildew species, primarily to perform phylogenomics analyses.

Associated personnel:
Sarah Hambleton, Christopher Lewis, Jeff Cullis – AAFC Ottawa
Guillaume Bilodeau – CFIA Ottawa
Tom Graefenhan – Canadian Grain Commission Winnipeg
Doug Luster, Doug Peterson – USDA-ARS FDWSRU
Lisa Castlebury – USDA-ARS Beltsville
Lori M. Carris – Washington State University

***Rathayibacter* species as a proposed model bacterial system for AAFC-ARS KBASE annotation.**

Rathayibacters are gram positive phytobacteria commonly associated with forage grasses. The most significant member of the genus is R. toxicus, a USDA-APHIS select agent responsible for a disease of livestock called Annual Ryegrass Toxicity (ARGT). ARGT occurs when *R. toxicus* produced toxins accumulate in seeds of forage grasses that are consumed by cattle and sheep, resulting in staggers symptoms and death. The genomes of six *Rathayibacter* species have been completed, but annotation has been tricky due to the fact that the GC content of this genus is high (~60%) and the lack of completely annotated related bacteria. The *R. toxicus* genome has been annotated using other software. Testing the KBASE annotation on the *R. toxicus* genome (as well as other toxin-producing and non-producing *Rathayibacter* species) would be a good test of the system's ability to handle novel bacteria.

Associated personnel:
William Schneider, Aaron Sechler, Doug Luster – USDA-ARS FDWSRU
Brenda Schroeder – University of Idaho
Tim Murray, Stacy Mauzey – Washington State University
Jim Stack, Mohammed Arif – Kansas State University
James Tambong, Christopher Lewis, Jeff Cullis – AAFC Ottawa
Sean Li – CFIA Charlottetown

File S3

USDA United States Department of Agriculture | Research Education Economics | Office of the Chief Scientist | Room 214-W Jamie L. Whitten Building Washington, DC 20250-0110

JUL 20 2018

Brian T. Gray, Ph.D.
Assistant Deputy Minister
Science and Technology Branch
Agriculture and Agri-Food Canada

Dear Dr. Brian Gray,

It was a pleasure to meet with you on June 18 in Washington, D.C. to discuss the shared research priorities between USDA and AAFC, and to follow up on the recent G20 Meeting of the Agricultural Chief Scientists (MACS). It is encouraging to see the tremendous progress our researchers achieve through working together. The final "June 2018 Progress Report on USDA-AAFC Cooperation in Agricultural Research and Development" is attached for your records.

During our discussion, we decided to graduate "Global Earth Observation" and rename "Agricultural Resilience" as "Agroecosystem Living Labs and Climate Partnerships." Thus, going forward, our five priority areas will be: agroecosystem living labs and climate partnerships; antimicrobial resistance; monitoring and genotyping of high risk plant pathogens; oat genetic improvement; and pollinator health. We also discussed the need to review "Antimicrobial Resistance" at the time of the next joint report, and to further explore whether "Genome Editing" may be useful to consider as a new priority area.

During our meeting, we also discussed formalizing what it means to be a USDA-AAFC collaborative priority area as well as how to determine when a priority is ready for "graduation." I look forward to the development of renewed guidelines and principles for joint research priorities going forward.

The Office of the Chief Scientist (OCS) will continue to coordinate USDA collaborations with AAFC on the strategic priority areas, with Genevieve Croft (genevieve.croft@osec.usda.gov) as the lead staff contact.

Sincerely,

Chavonda Jacobs-Young

Chavonda Jacobs-Young, Ph.D.
Acting Chief Scientist
Acting Deputy Under Secretary, USDA

Enclosure:
June 2018 Progress Report on USDA-AAFC Cooperation in Agricultural Research and Development

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada
Science and Technology Branch | Direction générale des sciences et de la technologie

LOB 243206

Chavonda Jacobs-Young, Ph.D.
Acting Chief Scientist
Acting Deputy Under Secretary, USDA
Room 214-W
Jamie L. Whitten Building
Washington, DC 20250-0110

Chavonda

Dear Dr. Jacobs-Young,

Thank you for your letter of July 20, 2018, and for hosting our recent meeting in Washington, D.C.

Your correspondence regarding our cooperation is greatly appreciated, and I can confirm that it accurately reflects the discussions we had on June 18 regarding the adjustments to our priority areas. I have been pleased to see the high level of collaboration regarding "Global Earth Observation", and I am confident that those partnerships will continue to flourish. I look forward to reviewing progress in "Antimicrobial Resistance" and exploring "Genome Editing" as a new potential priority area in the future. I also support the renewal of the guidelines and principles for joint research priorities.

During our meeting, we also discussed our joint leadership of the Agroecosystem Living Labs (ALL) Working Group formed at the recent G20 Meeting of the Agricultural Chief Scientists (MACS). I welcome the opportunity to work together and leverage our respective capacities to advance the ALL approach on a global level as well as in our own countries.

I would also like to thank your USDA colleagues for the engaging discussions on Indigenous agriculture. I appreciated learning about the experiences and work being done by various branches of the USDA in this area and look forward to having further exchanges in the future.

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Thank you again for capturing our shared understanding in your letter and in the finalized joint progress report. Our International Engagement Division will continue to coordinate with Genevieve Croft in the Office of the Chief Scientist regarding our bilateral cooperation in strategic priority areas.

Sincerely,

Brian T. Gray, Ph.D.
Assistant Deputy Minister
Science and Technology Branch
Agriculture and Agri-Food Canada

United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

October 29, 2018

Dr. Brian T. Gray
Assistant Deputy Minister, Science and Technology Branch
Agriculture and Agri-Food Canada
1341 Baseline Road, Floor 6, Room 330
Tower 5
Ottawa ON K1A 0C5
Canada

Dear Dr. Gray:

In 2013, Agriculture and Agri-Food Canada (AAFC) and the U.S. Department of Agriculture (USDA) identified next-generation sequencing technologies and applications for genotyping and monitoring quarantine and invasive species as a collaborative strategic priority area. The goal was to facilitate research between the two agencies that would result in faster and more accurate diagnostic methods for these pathogens; these advances would enhance monitoring efforts for the introduction and dissemination of plant pathogens and advance United States-Canada trade in agricultural products. At that time, an Action Plan was drafted for a period of 3 to 5 years.

Since the inception of this effort, there have been successful bilateral meetings and collaborative projects between U.S. and Canadian scientists in important topics such as graminicolous downy mildews, *Synchytrium endobioticum*, potato zebra chip, and stem rust of wheat. Scientists with the Agricultural Research Service (ARS), USDA's chief in-house scientific research agency, have made key contributions to these projects, including:

- ARS researchers in Beltsville, Maryland, provided herbarium samples, including rust from Barberry (*Berberis*) plants, ergot fungi and powdery mildews, for subsequent DNA extraction and sequencing at AAFC;
- AAFC and ARS scientists are contributing members of the virtual International Centre for Fusarium Research;
- AAFC is working on molecular characterization of *Synchytrium* specimens sampled from the USDA herbarium. Sequence data is being generated for each terrestrial *Synchytrium* species and its host from the herbarium material.

The first AAFC-ARS Action Plan has ended, and it is necessary to meet and discuss the development of a new Action Plan. This new plan would incorporate updated research priorities based on the fundamental principle that collaborative efforts between the AAFC, Canadian Food Inspection Agency, ARS, and USDA's Animal and Plant Health Inspection Service will be synergistic in safeguarding Canadian and U.S. agriculture.

The main goal of the new Action Plan will be to speed up the creation and adoption of technological innovation that use advancements in sequencing technology, bioinformatics, and artificial intelligence to prevent the introduction and spread of high-risk plant pathogens into Canada and the United States.

We look forward to discussing and formulating this new plan in more detail and to our continued cooperation on this research

Sincerely,

Chavonda Jacobs-Young

CHAVONDA JACOBS-YOUNG
Administrator