Diagnostic Services for Dickeya and Other Potato Pathogens

Mathuresh Singh
Agricultural Certification Services Inc.
1030 Lincoln Road
Fredericton, NB
Agricultural Certification Services

- Established in 1996
- CFIA approved
- Not for profit organization
- Owned by potato growers of NB
- Managed by Potatoes NB
Agricultural Certification Services

- Full time employees
  - Angela Gallagher
  - Megan Hampsey
- Project employees
  - Dr. Tyler MacKenzie
  - Dr. Manisha Shukla
- Casual employees
  - 7 to 10
Canadian Food Inspection Agency accreditation

• Quality Assurance manual (ISO 17025)
• Use protocols and controls (Positive and Negative) provided by CFIA
• Technicians have to do blind panels, twice a year to maintain their certification
• Submit positive samples for confirmation
• Daily monitoring of equipment
• Audit of the laboratory documents
• Annual certification of equipment by third party
Agricultural Certification Services

• Currently serving growers and institutions across Canada
• Work closely with provincial and CFIA certification staffs
• Research collaborations with NBDAAF, Agriculture & Agri-Food Canada, McCain’s, CHC and different grower organizations
Potato Seed Certification

• Visual inspection during growing season
• Laboratory testing
  – Nuclear stock
  – Export and Import requirements
## Tolerances (%)

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<th>N</th>
<th>PE</th>
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DISCLAIMER – for all lab tests

PLEASE READ CAREFULLY BEFORE SIGNING

The test results reported by Agricultural Certification Services are based only on the samples submitted for testing by the customer. The material tested has been submitted for testing by the customer and we take no responsibility for the test results other than in reporting those results to the customer.

Agricultural Certification Services makes no representations, guarantees or warranties expressed or implied, as to the test results. The customer further agrees to indemnify and hold harmless Agricultural Certification Services and/or Potatoes New Brunswick (NB Potato Agency), its directors, servants and agents from all actions, causes of actions, losses, damages, expenses and claims whatsoever against Agricultural Certification Services and/or Potatoes New Brunswick by reason of any claim by any person with respect to the use of these test results.

Potatoes NB and ACS Inc. reserves the right to share test results with all appropriate organizations as per federal/provincial regulations and/or industry requirements. Test results from Agricultural Certification Services laboratory will be released only to customers that are not in arrears with either Potatoes NB (NB Potato Agency) or ACS Inc.

I have read and understand the above disclaimer. I agree with the above statements. I declare that I am the owner of the sample or am acting as an agent of the owner of the sample.
Master Inventory

- ACS Number
- Test Reference
- Grower Name
- Variety
- Class
- Certification Number
- Date Received
- Number of bags
- Received by
- Discarded on & by
Approx. 8000 sq. ft.

Three different sections

• Receiving, coring, grinding and storage
• Laboratory and offices
• Greenhouses
ACS SERVICES

• BACTERIAL RING ROT TESTING
  – Testing of tubers or stems and nuclear stock plantlets for certification purposes and for export
  – TAS-ELISA & IMF
TESTING PROCEDURE

- ELISA test for screening (400 tubers are divided in groups of two 200 tubers)
- If positive then IMF (immuno-fluorescence) test done
- If IMF test positive (150 cells/30 counts)
- Submit sample to CFIA lab for confirmation and inform local CFIA field staff
- If results from both labs are positive then sample is declared positive and it triggers CFIA investigation
TESTING PROCEDURE

• ELISA test for screening
  400 tubers
  200  200
GRINDING PROCESS

No external contamination is detected.
Sap for ELISA
ELISA PLATE
ELISA PLATE READER
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Immuno-fluorescence Testing

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<th>DILUTIONS</th>
<th>COUNTS</th>
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<td>1:10</td>
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<tr>
<td>1:50</td>
<td>30</td>
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<tr>
<td>1:100</td>
<td>30</td>
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150 CELLS OR MORE – SUSPECT/POSITIVE
RESULT CONCLUSIONS

• If ELISA is positive perform IMF
• If ELISA is positive and IMF is negative - repeat IMF (by two technicians)
• If IMF is negative in second test, then sample is negative
ACS SERVICES

POTATO SPINDLE TUBER VIROID TESTING (PSTVd)

- Testing of nuclear stock and seed lots for export certification

- R-PAGE
ACS SERVICES

• VIRUS TESTING - NUCLEAR AUDIT
  – Testing of leaves, tubers, sprouts and plantlets for Potato Viruses A, M, S, X, Y, Potato Leafroll Virus, Potato Latent Virus and Potato Mop Top Virus
  – ELISA (DAS & TAS)
• VIRUS ERADICATION
  – For new varieties or new lines of existing varieties for entry into the Canadian seed potato certification program using Chemo and Thermotherapy
ACS SERVICES

• LATE BLIGHT TESTING
  – Testing for *Phytophthora infestans* in tubers or leaves using visual inspection after incubation at 16-18°C for 3 weeks with high humidity or by ELISA and PCR
ACS SERVICES

PINK ROT TESTING

Testing for *Phytophthora erythroseptica* in stems or tubers using PCR
ACS SERVICES

• DON TESTING (VOMITOXIN)
  – For the presence of Fusarium toxin in grains such as Wheat, Barley, Oat, Corn and their products
  – ELISA
ACoS SERVICES

Strawberry virus testing

• ELISA
  – *Strawberry mild yellow edge virus* (SMYEV)

• RT-PCR
  – *Strawberry mild yellow edge virus* (SMYEV)
  – *Strawberry mottle virus* (SMoV)
  – *Strawberry vein banding virus* (SVBV)
  – *Strawberry crinkle virus* (SCV)
  – *Strawberry pallidosis associated virus* (SPaV)
  – *Strawberry polerovirus 1* (SPV1)
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<th>Sample Description</th>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
<td>SMoV</td>
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<tr>
<td>3</td>
<td>SV BV</td>
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<tr>
<td>4</td>
<td>SMY EV</td>
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<tr>
<td>5</td>
<td>SMoV+SMY EV</td>
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<tr>
<td>6</td>
<td>SMY EV+SV BV</td>
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<td>7</td>
<td>Composite sample</td>
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<td>8</td>
<td>Plant A - SV BV</td>
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<td>9</td>
<td>Plant B - SMoV+SMY EV</td>
</tr>
<tr>
<td>10</td>
<td>Plant C - virus free</td>
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- **Simplex RT-PCR**: Targeting single viruses
- **Duplex RT-PCR**: Targeting two viruses simultaneously
- **Quadriplex RT-PCR**: Targeting three viruses and an internal control mRNA simultaneously

- SV BV 544bp
- SMY EV 383bp
- SMoV 246bp
- Control 85bp
ACS SERVICES

- POST HARVEST VIRUS TESTING (PHVT)
  - For mosaic (PVY), Potato leafroll virus (PLRV), PVA, PVS and PVX in seed potatoes prior to export, import or planting
  - ELISA & PCR
• **PVY strain differentiation**
  - PVY $^o$
  - PVY $^{N:O}$
  - PVY $^{NTN}$
Detection of PVX, PVY, PLRV, PVA and PVS using Pentaplex RT-PCR format
MULTIPLEX SYSTEMS

SHEPODY


NUMBER OF CYCLES

DELTA Rn

1 PVY #1-NED NED
1 PLRV-FAM FAM
1 PVS#2-TET TET

Potatoes

Agricultural Certification Services | Services de Certification Agricole
ACS SERVICES

CONDUCTING SCIENTIFIC RESEARCH

– Development of diagnostic procedures for the detection of different pathogens
– Current season spread and Management of PVY
DICKEYA & PECTOBACTERIUM TESTING

Testing in stems, tubers and water using PCR


3. Dr. Gary Secor, North Dakota State University
A large sample of tubers (200-400 from each lot) has their stem-end removed as a small (~1cm) piece using potato peeler. Cores are grouped into beakers as 25 core composite samples.
In the beakers, composite samples are washed briefly, then dried.
Cores from each beaker are transferred to heavy-duty sealed plastic bags to be pulverized for releasing the bacteria from the potato tissue.
Sap from pulverized cores are transferred to large vials for freezer storage (below), and small subsamples are added to tubes of buffer for DNA extraction and tubes of glycerol stabilize the live bacteria for freezer storage (right).
Applying stabilized bacteria to semi-selective media to preferentially culture Dickeya and similar pectolytic bacteria.
Dickeya Extraction Methods

During development of our Dickeya detection protocols, we investigated a wide range of DNA extraction techniques.

Samples tested included a **known positive tuber** treated several ways:

a. Macerated tuber in water
b. Macerated tuber in water incubated 24h at 30°
c. Washing water from intact tuber core incubated 24h at 30°
d. Negative control tuber not infected with Dickeya
Dickeya Extraction Methods

During development of our Dickeya detection protocols, we investigated a wide range of DNA extraction techniques:

1. Simple boiling water extraction (STANDARD)
2. Qiagen Plant Mini kit
3. Qiagen Bacteria/yeast kit
4. Boiling extraction with alcohol precipitation
5. Classic strong phenol-chloroform extraction
6. MoBio Soil DNA kit
7. MoBio Plant DNA kit

[Image: DNA gel electrophoresis results with lanes labeled 1 through 7, including a control lane labeled 'controls'.]
Dickeya Extraction Methods

During development of our Dickeya detection protocols, we investigated a wide range of DNA extraction techniques. The most standard method of the boiling water extraction works adequately, and better than most others.

While the Qiagen Bacteria/Yeast kit seemed to give higher yield, for the added expense and processing time, it was not a better indicator of Dickeya presence.
Primers used for PCR

Dickeya spp.
-Pel ADE
-ECH
-DF/DR

D. dianthicola
-DIA & DIC
PCR GEL
DNA alignment based on 16S ribosome sequence.

This sequence is very conserved across species (RED)...

...but characteristic sequence differences (BLUE) allow matching of sample DNA to known species.

Sequences here:
ACS-collected sample
D. dadantii reference
D. solani reference
D. zeae reference
D. dianthicola reference
<table>
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<tr>
<th>Lab</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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Lab 1. Conventional PCR ADE primers 35 cycles

Lab 2. Conventional PCR ADE primers 30 cycles. Same results Flic primer but I neg; probably not dianthicola

Lab 3. Conventional PCR same results with ADE or ECH primers

Lab 4. Conventional PCR ADE primers; same results with 25 or 35 cycles.

Lab 5. Real time PCR Df/Dr primers 34 cycles

Lab 6. Conventional PCR ADE primers 25 cycles
QUESTIONS?