DNA Nultiscan[®]: A New Tool for Rapid Detection of Pathogens in Water, Soil, and Plant Tissue[®]

Marc Sabourin

University of Guelph, Laboratory Services, 95 Stone Road, West Guelph, Ontario, Canada N1H 8J7

Email: msabouri@isd.uogeulphca

The main limitation in plant disease management is the ease of plant pathogen identification. Standard procedures' limitations include:

- Time-consuming and often laborious.
- Require extensive knowledge in both classical taxonomy and culture methods.
- Exclude culture-independent organisms.
- Detect few organism at a time.

Molecular and serological identification methods, on the other hand, generate accurate results rapidly but detect few organisms at a time. The solution is DNA-array technology:

- Originally developed to screen for human genetic disorders in 1989.
- Successfully applied to detect and identify different microorganisms in clinical laboratories in 1992.
- Successfully applied to discriminate and identify DNA samples isolated from specific oomycete (1998), nematode (1999), and bacterial cultures in plant pathology (2003).

The advantages of DNA Multiscan include:

- Multiplex detection.
- Rapid, accurate, simple, and sensitive.
- Semi-quantification.
- Analysis of samples from different biological sources (plants, seeds, soils, composts, potting mixes, rockwool, water, nutrient solution, etc...).
- New microorganisms added regularly.
- Bundling of organisms to meet needs.

Currently detectable organisms are shown in Table 1.

During the analysis, the sample's total DNA is extracted. This includes any plant DNA, bacterial DNA, fungi DNA, yeasts DNA, algae DNA, and nematode DNA. Amplification of pathogens DNA is then carried out. This is followed by hybridization, which is a key step of the DNA Multiscan process. Keys to the success of the process are:

- The specific labeled amplified DNA sequence hybridizes with its correspondent oligo on the macro-array.
- The oligo is specific to the disease causal agent and can be developed in-house for specific needs.

Table 1. Currently detectable organisms by DNA Multiscan®.

FUNGI AND OOMYCETES:

Athelia (Sclerotium) rolfsii Phytophthora fragariae
Botrytis cinerea Phytophthora infestans
Colletotrichum sp. Phytophthora nicotianae
Colletotrichum acutatum Phytophthora ramorum
Colletotrichum coccodes Phoma destructiva

Colletotrichum fragariae Plectosphaerella cucumerina Colletotrichum gloeosporioides Pyrenochaeta lycopersici

 $Colletotrichum\ graminicola$ $Pythium\ sp.$

Cylindrocarpon destructans Pythium aphanidermatum Cylindrocladium sp. Pythium dissotocum Didymella sp. Pythium irregulare Fusarium sp. Pythium polymastum Fusarium oxysporum Pythium sylvaticum Fusarium solani Pythium ultimum Gnomonia comari (Zythia fragariae) Rhizoctonia solani Penicillium sp. Sclerotinia sp.

Phytophthora sp. Sclerotinia minor
Phytophthora cactorum Sclerotinia sclerotiorum
Phytophthora capsici Sclerotinia trifoliorum
Phytophthora cinnamomi Thielaviopsis basicola
Phytophthora citricola Verticillium sp.

Phytophthora cryptogea Verticillium albo-atrum Phytophthora drechsleri Verticillium dahliae

BENEFICIALS:

Trichoderma asperellum Trichoderma harzianum Trichoderma hamatum

BACTERIA:

Erwinia carotovora subsp. atrospetica

Erwinia carotovora subsp. carotovora

Erwinia chrysantemi

Pseudomonas cichorii

Pseudomonas marginalis

 $Pseudomonas\ viridiflava$

Pseudomonas syringae pv. porri

Ralstonia solanacearum

Rhizobium radiobacter (syn. Agrobacterium tumefaciens)

Xanthomonas fragariae

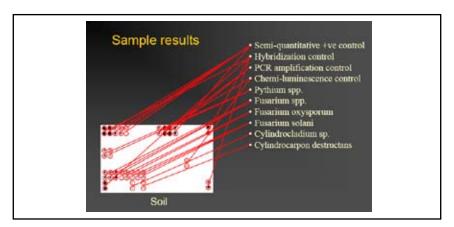


Figure 1. Example of sample results.

An example of sample results is shown in Fig. 1.

Current applications include the following:

- Disease diagnosis.
- Continuous monitoring recirculating greenhouse fertilizer solutions or pond water.
- Detection of organisms in soil, peat, compost, and other growing media.
- Regulatory requirement to justify the use of certain restricted chemical pesticides.