



Array-Technology in Potato Pathogen Detection

PROJECT COST OC 853.002

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Investigated viruses

- Potato virus A (PVA) *Potyviridae*
- Potato virus S (PVS) *Carlavirus*
- Potato virus X (PVX) *Potexvirus*
- Potato virus Y (PVY) *Potyviridae*
- Potato virus M (PVM) *Carlavirus*
- Potato leaf-roll virus (PLRV) *Poleovirus*
- Potato Mop-top virus (PMTV) *Pomovirus*

Characteristics

- + ssRNA viruses
- 6 000 - 10 000 bp
- helical symmetry of capsid
- propagation by insects (except PVX)
- mixed infections

Symptoms of PLRV



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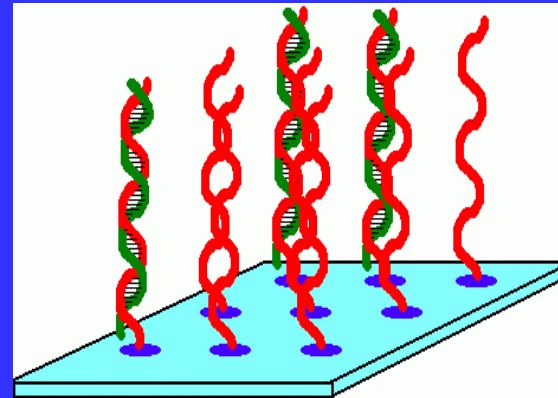
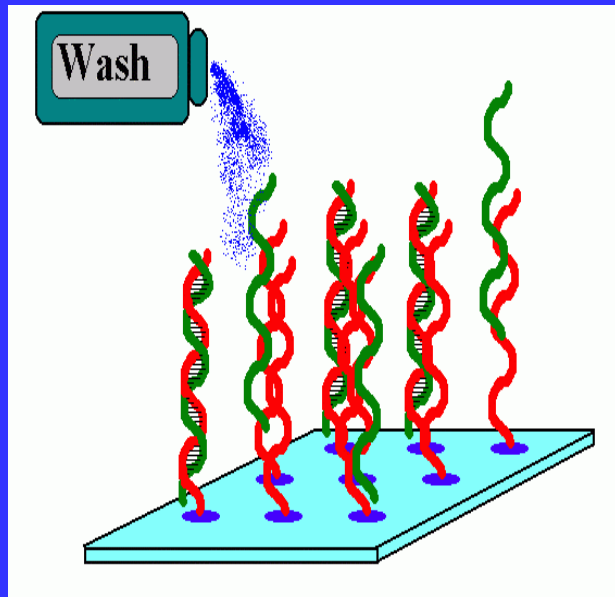
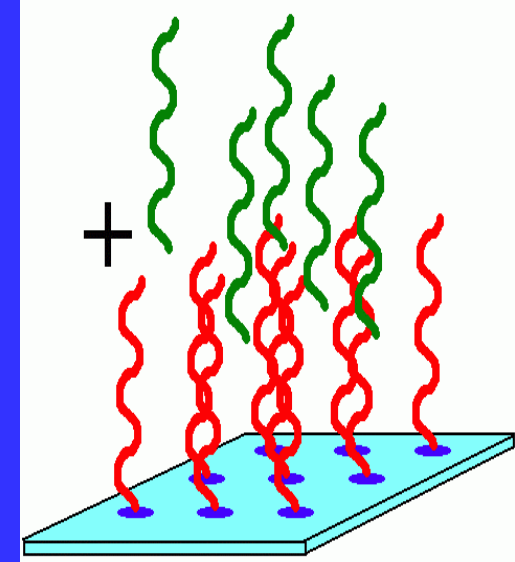
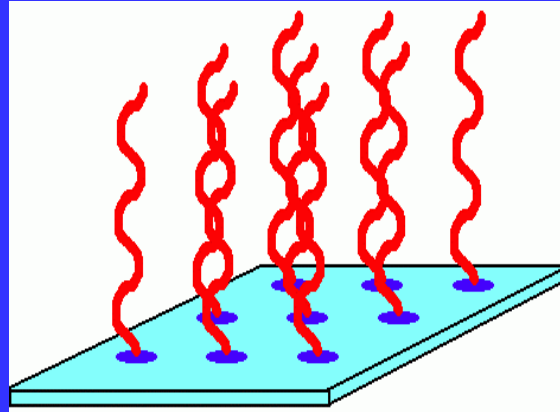
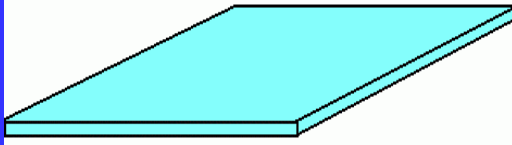
Symptoms of PVY



Main approaches

- RT-PCR detection
- multiplex RT-PCR
- ELISA
- „Macroarrays“ – classical hybridization
- Microarrays

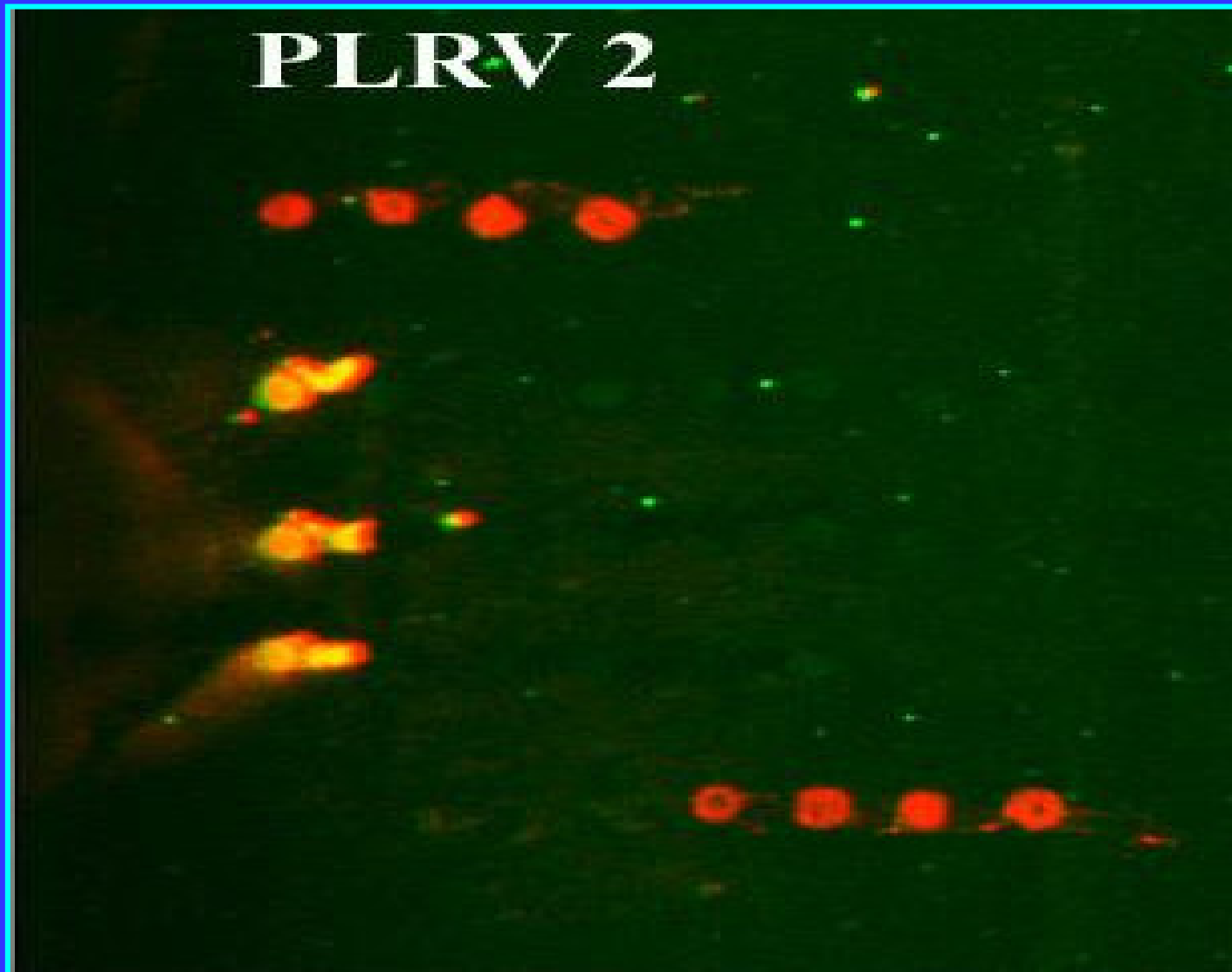
Experimental



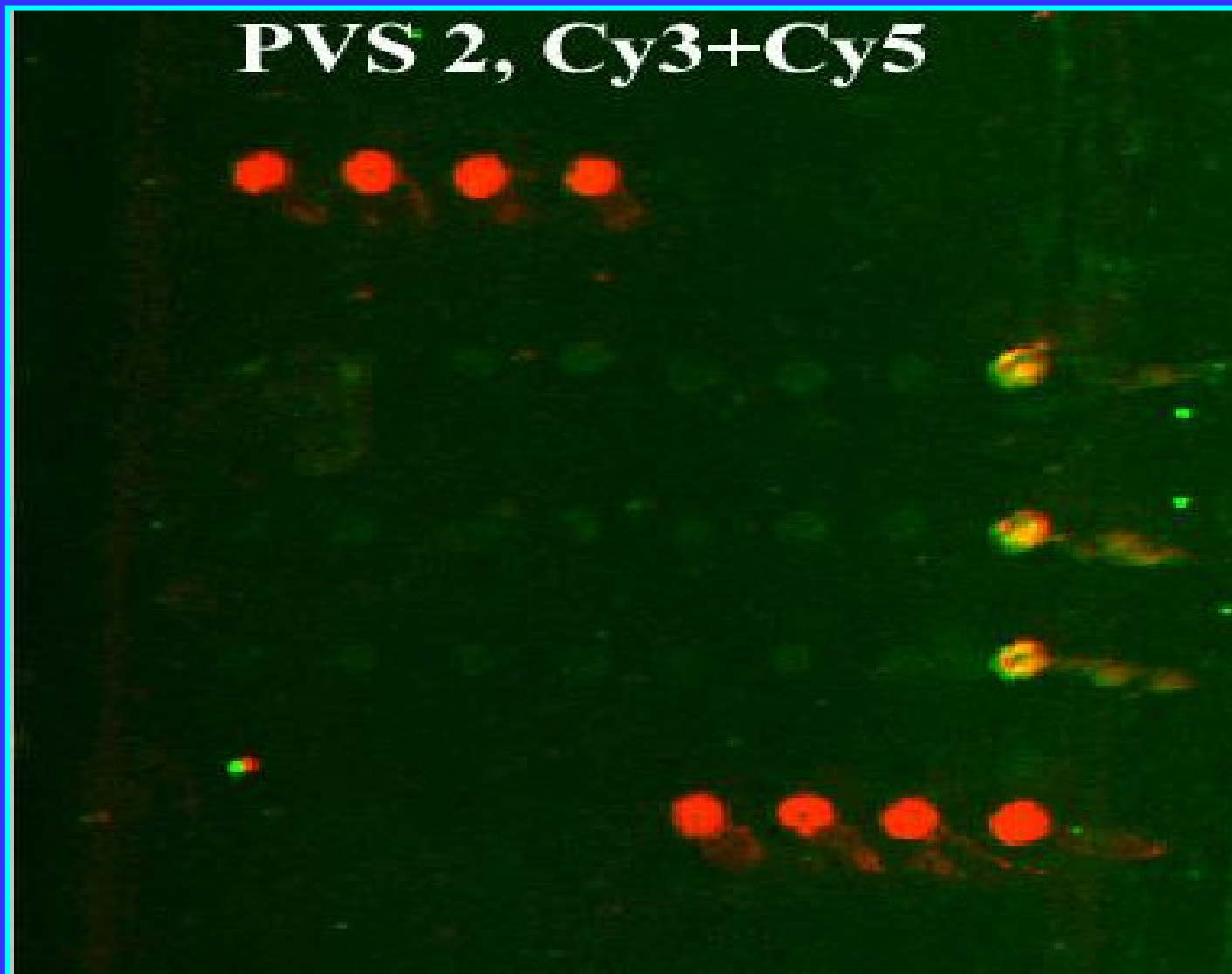
Preparation with cloned sequences

- RNA isolation
- RT-PCR
- Purification of amplicons
- Cloning
- Spotting on glass

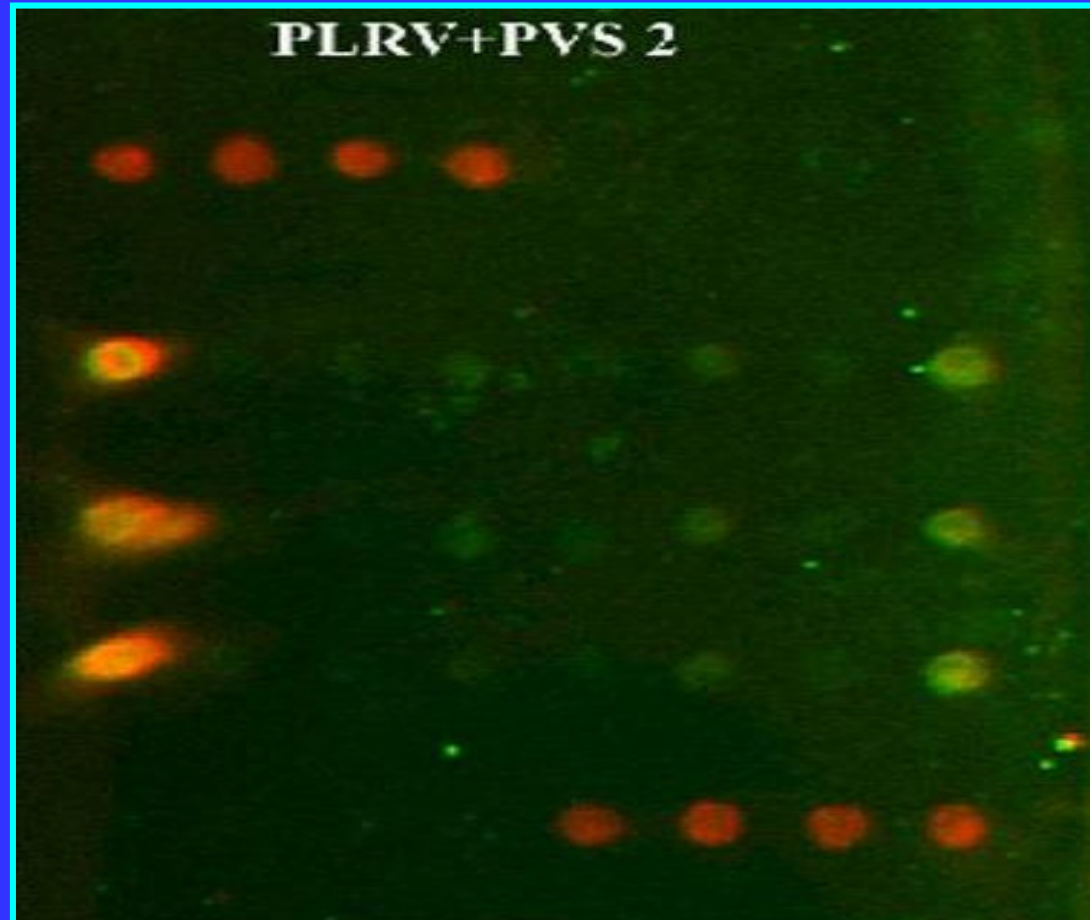
Detection



Detection of PVS



Parallel detection of viruses



red: standard targets hybridized with standard probes (positive control of hybridization)

orange: PLRV (targets) hybridized with specific probes

green: PVS (targets) hybridized with specific probes

Published in:

- Bystricka D., Lenz O., Mraz I., Dedic P. and **Sip M.**: DNA Microarray: Parallel Detection of Potato Viruses, Acta Virologica 47, 2003, p. 45-48

Short oligonucleotide approach

- short synthetic single stranded oligomers (40 nt)
- length 40 nt , melting temperature 60 - 65°C, $\Delta G < -65$ kcal/mol (Vector NTI Suite, InforMax)
- simultaneous detection of seven potato viruses (PVA, PVS, PVM, PVX, PVY, PLRV and PMTV)
- able to distinguish PVY^{NTN} and PVY^O strains
- at least 4 probes for each of virus, according to results obtained from BLAST

(Results in press)

Collaborations

- University of South Bohemia
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 - Dasa Bystricka
 - Marie Vokurkova
- Institute of Plant Molecular Biology, České Budejovice
 - Ivan Mraz
 - Ondrej Lenz
- Center for Inherited Metabolic Diseases , Prague
 - Stanislav Kmoch
 - Martin Hrebicek
- Potato Research Institute, Havlickuv Brod
 - Petr Dedic

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