

Construction of an oligonucleotide microarray for detection and identification of the potato wart fungus *Synchytrium endobioticum* and viral pathogen sequences in potato

Ismail Abdullahi & Stephan Winter

German Collection of Microorganisms and Cell Cultures /DSMZ) Plant Virus Department, Braunschweig, Germany



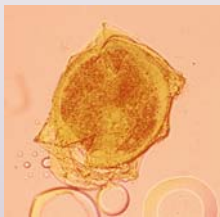
Sequences from viruses and other pathogens infecting potato were screened for oligonucleotide sequences indicating for species/ type or strain that can be utilised in a general potato pathogen diagnostic chip developed by the EU Framework 5 DIAGCHIP consortium.

This study was, to evaluate whether informative sequences of *Synchytrium endobioticum*, the causal agent of the quarantine potato wart disease, can be obtained and employed for reliable identification of fungal sequences in a DNA microarray.

Isolation of DNA from resting spores

Resting spores were isolated from infected potato gall wart tissue and examined for morphological identification. After fractionation through 75 and 38 μm sieves, sucrose centrifugation and surface sterilisation, DNA was extracted from spore suspensions using the DNeasy protocol (Qiagen, Germany).

Mature resting spore of *S. endobioticum* isolated from wart infected potato tubers.



Analysis of 18 rDNA sequences, oligonucleotide design and synthesis

The nt sequence for the 18S rRNA gene was generated by PCR amplification using universal primers (Simon et al. 1992, *Appl Environ Microbiol* 58(1):291-295). Clustal X alignment of approx. 1700nt 18S rRNA sequences revealed a high sequence conservation among related and non related fungal species (yellow) but also significant and long stretches of sequence with high diversity (blue). These regions were chosen best for design of the target sequences.



Clustal X multiple sequence alignment of 18S rDNA sequences from *Synchytrium endobioticum* and several fungal species, to reveal discriminative sequence patterns (blue). Arrow indicates for probe position with best array performance.

Synchytrium endobioticum strain 58; EF426540, *Plasmopara viticola*; EF418924, *Pythium oestracodes*; AY742758, *Pythium vexans*; DQ322623, *Synchytrium macrosporum*; AY546682, *Monoblepharella* sp.; AY546683, *Cladochytrium replicatum*; AF164335, *Monoblepharella elongata*; M59759, *Spizellomyces acuminatus*; M59761 *Neocallimastix* sp.

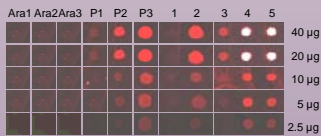
RNA isolation, probe labelling and hybridization

RNA was extracted from healthy and wart-infected potato tubers and from virus, PBRSV, PVS, APLV, APMV, TSV, PVA, PVY, PVV, PVX and PSTV infected leaf tissues, 1:10 (w/v) in RNAwiz (Ambion, Huntingdon, UK). The CyScribe First-Strand cDNA Labelling Kit (Amersham) was used for cDNA synthesis with anchored oligo-dT and random nonamers. For hybridisation to microarray slides, Cy3 and Cy5 labelled probes were combined, denatured at 95°C for 10 min kept and directly added to pre-heated (55°C) microarray hybridization buffer (MWG) for 20 h hybridisation.

Oligonucleotide design and synthesis

In the DIAGCHIP project, oligonucleotides (50 mer) slides were designed for optimal and uniform hybridization kinetics and arrayed on epoxy coated glass slides at MWG, Ebersberg, Germany. In total, 180 features (spots) were printed in duplicate in 4 blocks, with 10 columns and 9 rows.

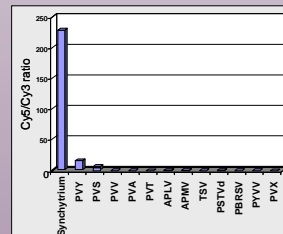
Intensity of microarray signals with amounts of total RNA labelled



Synchytrium endobioticum

2.5 μg , 5 μg , 10 μg , 20 μg and 40 μg RNA from wart potato tubers were separately labelled and used to hybridize 4 separate but similar microarrays. Ara1, Ara2 and Ara3, oligonucleotides for *Arabidopsis thaliana* genes (negative controls); P1, P2 and P3, oligonucleotides for NADH-ubiquitous oxidase, mitochondrial ATPase and 18S rRNA of potato/tobacco (positive controls).

1, 2, 3, 4 and 5 are probes to hybridize with 18S rDNA of *Synchytrium endobioticum*. Probe 2 position is indicated by an arrow in the S. sequence alignment.



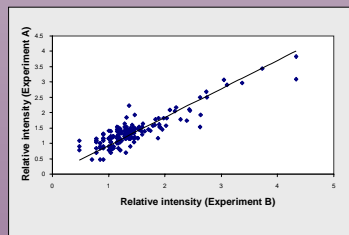
Specificity of *S. endobioticum* oligonucleotide probe 2

Total RNA from *Synchytrium*-infected potato tuber was labelled with Cy5 while RNA from healthy control plant was labelled with Cy3. Ratio (Cy5/Cy3) of median pixel intensities (background subtracted) of fluorescence after hybridizing microarray was plotted.



Andean potato latent virus

Approximately 0.5 μg , 1.5 μg , 3.0 μg and 6.0 μg RNA from (APLV) infected tobacco plant were treated as above. 6,7,8, and 9 are probes for specific detection of APLV.



Reproducibility of fluorescent intensities of *S. endobioticum* replicate probes within an array.

The logarithm of normalized median pixel intensity (minus background) from microarray hybridised with *S. endobioticum* target in one experiment (Experiment A) were plotted against the intensities for the same spots (probes) with another *S. endobioticum* target (Experiment B).



Image map of an oligonucleotide array hybridized with dye-labelled cDNA of *Synchytrium endobioticum* infected potato

Two (boxed) of five oligonucleotide probes reveal specific hybridisation signals with *S. endobioticum*.



Image map of an oligonucleotide array hybridized with dye-labelled cDNA of Andean potato mottle virus infected tobacco.

One (boxed) of five APMV probes reveals specific hybridisation signals with the APMV oligonucleotide.

The potential of an oligonucleotide-based microarray for identification of plant viruses is proven. For *Synchytrium endobioticum*, the sequence of the ribosomal 18S DNA permitted the selection of 50 nt oligomers with one being highly specific and sensitive for detection of pathogen sequences in cDNA from wart infected potato tubers using the DNA chip. This was possible with all four pathotypes tested.

This work was part of the EU IDAGCHIP project to investigate the feasibility of a diagnostic chip for the simultaneous detection of all the plant pests/pathogens present and stated in the EU Plant Health Directive (77/93/EEC).