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GENOMICS AND PROTEOMICS

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The 18S rDNA sequence of *Synchytrium endobioticum* and its utility in microarrays for the simultaneous detection of fungal and viral pathogens of potato

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Abstract Resting spores extracted from wart (*Synchytrium endobioticum*)-infected potato tubers were used for DNA extraction and amplification of 18S rDNA. Analysis of the cloned, sequenced fragment revealed high similarity to members of the Chytridiomycota. Using this information, specific oligonucleotide probes were designed and arrayed onto glass slides for detection of the pathogen. Viral sequence information available in the databank was retrieved, or new viral sequences were generated, and used to design probes for specific detection of important quarantine viruses of potato. To determine the sensitivity and specificity of the oligonucleotide probes, total RNA from infected plants was reverse transcribed, labelled with Cyanine 5, and hybridised with the microarray. A significant number of the oligonucleotide probes exhibited high specificity to *S. endobioticum*, *Andean potato latent virus*, *Andean potato mottle virus*, *Potato black ringspot virus*, and *Potato spindle tuber viroid*. Hybridisation signals of sub-arrays within slides were reproducible (r=0.79) with a high correlation coefficient of hybridisation repetitions (0.73). Our results demonstrate the potential of microarray-based hybridisation for identification of multiple pathogen targets, which will find application in quarantine laboratories, where parallel testing for diverse pathogens is essential.

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Introduction

Viruses and fungi are major groups of pathogens infecting potato (*Solanum tuberosum* L.). *Synchytrium endobioticum* (Schilberszky) Percival, a member of the Chytridiales in the phyllum Chytridiomycota, is the causal agent of potato wart disease, and is the most important quarantine fungus infecting potato. Members of Chytridiales have no hyphae, but rather sporangia that produce motile zoospores. *S. endobioticum* is one of the few species of chytrids of known economic importance, and is one of over 150 described species of *Synchytrium* known to cause significant economic losses in crop plants (Longcore 2001). Its principal host is potato but it also infects root tissues of tomato (*Lycopersicon esculentum* Mill.) and other solanaceous species without inducing gall formation. This fungus occurs worldwide, with reports of at least 30 pathotypes, defined according to their virulence on different potato cultivars (Baayen et al. 2001; Potocek 1977; Langerfeld et al. 1994; Melnik and Malakhanova 1998).

Because of the destructive nature of wart disease, *S. endobioticum* is considered an important quarantine pathogen worldwide (Smith et al. 1997). It produces resting spores, which can remain viable in the soil for a period of 30–70 years (Pratt 1975; Laidlaw 1985; Hampson 1993). The fungus has a limited capacity for natural spread, hence control by statutory measures has hitherto proved successful (Noble and Glynne 1970). However, reports of either re-emergence of erstwhile controlled pathotypes or emergence of distinct forms of the fungus (Stachewicz 1999) emphasise the need for constant monitoring of its incidence using efficient diagnostic tools.

In addition to potato spindle tuber viroid (PSTVd), at least 30 different viruses and several phytoplasmas are known to infect potato, causing significant damage (Salazar 1999). Potato leafroll virus (PLRV), *Potato virus Y* (PVY), *Potato virus X* (PVX), *Potato virus M* (PVM), and *Potato virus S* (PVS) are the most important potato viruses worldwide. Some strains of these viruses, which have distinct virulence features, are of restricted distribution; the two most important—Andean potato latent virus (APLV) and

Andean potato mottle virus (APMV)—are endemic in the Andean region. Rapid and accurate identification of these and other pathogens are key elements for the implementation of statutory measures aimed at preventing spread into non-endemic regions of the world.

Nucleic-acid-based detection of pathogens can be designed to target single pathogens or a group of pathogens by selecting highly specific, differential sequences or by choosing sequences that are conserved within a pathogen group or taxon. The simultaneous detection of all target sequences is possible by the application of microarray analysis, a technique that has emerged as a powerful and flexible procedure for analysing large numbers of nucleic acid fragments in parallel (Maskos and Southern 1992; Guo et al. 1994; Schena et al. 1996). This procedure, which combines nucleic acid hybridisation and fluorescence microscopic techniques, was originally designed for gene expression analysis and has now been applied to diagnosis of plant diseases, including those with viral etiology (Boonham et al. 2003; Lee et al. 2003).

A requirement for the application of microarray techniques to pathogen identification is a knowledge of genome sequences. Complete genome sequences for a number of viral pathogens and their strains are available, and provide the basis for selection of adequately conserved or differential probes. For some fungal pathogens, specific genes have been cloned and sequenced, opening the way for the application of DNA-based detection methods. The 18S ribosomal DNA is particularly useful, because of its high sequence conservation among related species. No sequence information is available for *S. endobioticum*, thus we decided to analyse its 18S rDNA, and to define sequences that can be used in the detection of the fungus in parallel with other viral pathogens of potato. This work forms part of a European Union project designed to investigate the feasibility of producing a diagnostic chip for the simultaneous detection of all plant pests/pathogens present in European Union Plant Health Directive 77/93/EEC.

Materials and methods

Resting spore isolation and DNA extraction

Resting spores were isolated from wart potato tissues, using the extraction method of Hampson et al. (1997) with modifications. Infected potato tissues were obtained from the collection of the Institute for Plant Protection in Field Crops and Grassland (BBA, Kleinmachnow, Germany). Gall wart was excised with a sterile scalpel, sonicated for 10 min in sterile distilled water containing 0.1% Triton X-100 and dried at room-temperature for 24 h. Tissues were soaked in physiological saline (85% NaCl) for 18 h then homogenised in saline. The homogenate was filtered through 75-µm and 38-µm sieves and resting spores were washed from the lower sieve into fresh saline. The spore suspension was layered onto an 80% (w/v) sucrose solution, centrifuged at 2,000 *g* for 10 min and washed in four changes of saline. To surface-sterilise spores, they were treated with 95% ethanol, Javex bleach, 2% Chloramine T in 0.1% Tween 20 for 10 min, and 0.2% streptomycin. DNA was extracted from spores using DNeasy, according to the manufacturer's instructions (Qiagen, Hilden, Germany). Spores were first mixed with quartz sand (Riedel-de Haen, Seelze, Germany) and ground in liquid nitrogen.

Amplification, cloning and sequencing of the 18S rDNA

DNA extracted from resting spores was used for amplification of 18S rDNA sequences using universal primers NS1 and NS6 (Simon et al. 1992). The reaction mixture, prepared in a total volume of 50 µl, consisted of 25 µl premixed 2× PCR buffer (MasterAmp PCR Optimisation Kit, Epicentre, Madison, Wis.), 2 µM each of the two primers, 5 U *Taq* polymerase and 10 µl DNA. The reaction mixture was subjected to the following temperature cycle: an initial denaturation at 95°C for 3 min followed by 35 cycles of 95°C, 1 min, 53°C, 1 min 30 s and 72°C, 1 min 30 s, and a final extension of 10 min at 72°C terminating amplification. Control reactions without DNA were included in each PCR experiment. Amplicons were excised and purified using a DNA gel extraction kit (Qiagen), ligated into the pDrive cloning vector (Qiagen), and subsequently transferred into *Escherichia coli* DH5α cells (Gibco-BRL, Paisley, UK). Ligation, bacterial transformation and plasmid preparation were performed according to standard protocols (Sambrook et al. 1989). For sequence analysis of plasmid mini-preparations carrying appropriate PCR fragments, the services of a commercial company (Sequiserve, Vaterstetten, Germany) were employed. Blast search was performed to confirm sequence identity.

RNA isolation

RNA was extracted from both healthy and wart-infected potato tubers. Tubers were lyophilised and ground in a sterile mortar before adding 1:10 (w/v) RNA isolation reagent, RNAwiz (Ambion, Huntingdon, UK). All other steps were performed as described by the manufacturer. The viruses potato black ringspot virus (PBRSV), PVS, APLV, APMV, *Tobacco streak virus* (TSV), *Potato virus A* (PVA), PVY, *Potato virus V* (PVV), PVX, and the viroid PSTVd, were extracted from their respective inoculated test plants (mainly Solanaceae) using RNAwiz according to the manufacturer's instructions. All virus isolates used in these studies were from the DSMZ Plant Virus Collection (Braunschweig, Germany). The quality and integrity of RNA was assessed by agarose gel electrophoresis and concentrations were measured spectrophotometrically.

Oligonucleotide design and synthesis

Oligonucleotides (50-mers), which were designed for optimal and uniform hybridisation kinetics, were amine-

modified before arraying on epoxy-coated glass slides (MWG Biotech, Ebersberg, Germany). In total, 180 features (spots) were printed in duplicate in four blocks, each block with ten columns and nine rows. The oligonucleotides were named a110boon01–a120boon119, and are referred to as such in this paper. Specific probes for the 18S rDNA of the fungus *Synchytrium endobioticum* and 11 quarantine viruses of potato were arrayed. Ten oligonucleotides were designed as positive controls, targeting different genes of *Solanum tuberosum*. Three *Arabidopsis*-specific sequences were also included to serve as negative controls. Probes specific for bacteria and nematode species that are of quarantine interest in potato were also arrayed, although this work was not designed to test the specificity of these latter probes.

Probe labelling and hybridisation

The CyScribe First-Strand cDNA Labelling Kit (Amersham, Little Chalfont, UK) was used to prepare labelled cDNA. Anchored oligo-dT and random nonamers were used as primers. Each primer (1 μl) was mixed with 5, 10 or 30 μg total RNA in a total volume of 11 μl and incubated at 70°C for 5 min. The reaction was kept for 10 min at room temperature and the following components were then added on ice: 5× CyScript buffer (4 μl), 0.1 M DTT (2 μl), dCTP nucleotide mix (1 μl), dCTP Cyanine 3/Cyanine 5 (Cy3-/Cy5)-labelled nucleotide (1 μl) and CyScript reverse transcriptase (1 μl). RNA from healthy and infected plants was labelled with Cy3 and Cy5, respectively. The reaction was incubated at 42°C for 1.5 h and the un-reacted RNA was degraded using NaOH. The reaction was stopped with HEPES, labelled cDNA was purified (QIAquick PCR purification kit, Qiagen), then precipitated with alcohol. The amounts of Cy3 or Cy5 dye incorporated into cDNA were measured (Novaspec II spectrophotometer, Amersham Biosciences) at 550 and 650 nm, respectively, before and after purification of the labelled cDNA.

After re-suspending in 10 mM EDTA, the Cy3- and Cy5-labelled probes were combined, denatured at 95°C for 10 min, then immediately kept on ice for 30 s. Pre-heated (55°C) microarray hybridisation buffer (25 μl; MWG) was added and the mixture applied to the microarray slide. The slide was kept in a humid chamber for about 23 h at 55°C for the probe to hybridise, and was washed and dried with a gentle air stream before scanning.

Scanning and data processing

Hybridised arrays were scanned with a GMS 418 Array Scanner (Genetic MicroSystems, Woburn, Mass.). Laser lights of wavelengths at 532 and 635 nm were used to excite Cy3 and Cy5 dye, respectively. The laser power and photomultiplier tube gains were set to minimise features being saturated, while at the same time providing sufficient fluorophore excitation for adequate signal-to-background performance. Fluorescent images were captured as multi-image-tagged image file format and transferred to GenePix Pro 4.0 (Axon Instruments, Union City, Calif.) for analysis. The extent of hybridisation in positive samples was determined from the median value of pixel intensity. To compensate for variations in sample treatment, labelling, dye efficiency and detection, which potentially affect the final feature intensity, data were subjected to normalisation before analysis.

A simple global scaling procedure was applied, in which pixel intensity of each feature was multiplied by a constant factor so that the mean intensities of the arrays to be compared were identical. For interpretation of results we used the ratio of median pixel intensity (minus background) in infected vs healthy samples. We arbitrarily chose a ratio of ≥2.5; any hybridisation signal exceeding this threshold was regarded as positive.

Results

A contiguous nucleotide sequence of 1,421 bp (EMBL accession number AJ784274) was obtained after sequencing the 18S rDNA amplicon of *Synchytrium endobioticum*. A Blast search of this sequence in the NCBI database showed high similarity to members of the Chytridiomycota in the order Chytridiales, Neocallimasticales, and Spizellomycetales. When optimal alignment (Clustal W; Thompson et al. 1994) of the *Synchytrium endobioticum* sequence with other Chytridiomycota was made, an insertion of about 440 bp at position 540 was observed in *Spicellomyces punctatus*. Such long insertions, sometimes up to ca. 1,500 nucleotides, have been previously used in other fungal groups to indicate the presence of introns (Gargas et al. 1995). *Neocallimastix* species and *Synchytrium* contain 103 bp and 129 bp insertions, respectively, within this region, sharing no similarity. Hence this region could be used to design oligonucleotide probes to discriminate these species.

Specificity of the oligonucleotide probes

To test the specificity of the oligonucleotide probes against other fungal pathogens, total RNA extracted from mycelium of *Alternaria solani* and *Sclerotinia sclerotiorum* was labelled and hybridised to the microarray. One of the five *Synchytrium endobioticum*-specific probes, a120boon093#1, cross-hybridised with *A. solani* but not with *Sclerotinia sclerotiorum*. There was no unspecific reaction with virus-specific probes, and the two *Synchytrium*-specific probes (a120boon092#1 and a120boon094#1) were highly specific, hybridising only to the target (Fig. 1a). When RNA from healthy potato tubers was labelled, positive hybridisation signals were obtained only for the positive control probes. The high specificity of the *Synchytrium*-specific probes (except a120boon093#1) was further demonstrated by the lack of hybridisation with a panel of viral gene sequences present on the microarray. Analysis of the Cy5/Cy3 ratio of medians (Fig. 2) further proved the spec-

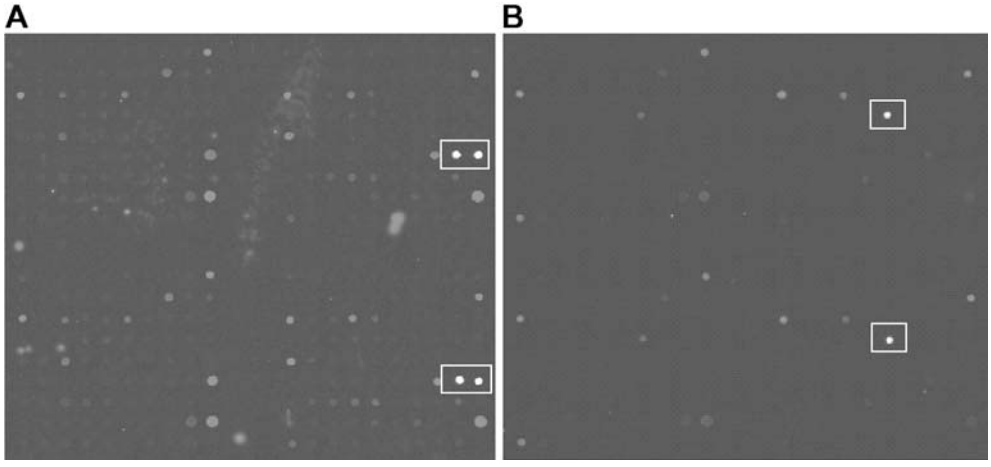

Fig. 1 Image map of an oligonucleotide array hybridised with dye-labelled cDNA of **a** *Synchytrium endobioticum*-infected potato and **b** Andean potato mottle virus (APMV)-infected tobacco. RNA was extracted from wart tissues (potato tubers) or leaves of APMV-infected tobacco, directly labelled with Cyanine 5 (Cy5) and used in microarray hybridisation. **a** Two (boxed) of the five oligonucleotide probes showed a specific hybridisation signal with *S. endobioticum*. Similar hybridisation patterns were obtained with fungal pathotypes 1, 2, 6 and 18. **b** One (boxed) of the five APMV-specific probes hybridised specifically with its target. Ratio of median =5.37

ificity of the reaction. Labelled cDNA targets from leaves and stems of wart-infected potato were also used for hybridisation, resulting in positive hybridisation signals only for the positive control (image map not shown). Labelled cDNA from APMV-infected plants (Fig. 1b) and from plants infected with a number of other viruses including APLV, PBRSV, and PSTVd, hybridised specifically to their respective target sequence.

Reactivity of the oligonucleotide probes

Assessing assay sensitivity is important since detection of pathogen is desired not only for cases of high but also of low pathogen abundance. The hybridisation signals obtained with positive controls were generally weak compared with signals generated from pathogen-specific probes (Fig. 3). High laser power was used to reveal even weak signals from the positive controls (Fig. 3a, b; P1–P3). This resulted in saturation of signals obtained with other targets (Fig. 3a, 4,5; Fig. 3b, 6–9). To determine detection levels and thresholds, a decreasing amount of total RNA was transcribed into labelled cDNA and hybridised to the microarray. Specific hybridisation signals were obtained when as little as 2.5 μg total RNA from wart-infected potato tubers (Fig. 3a) was labelled. However, the ratio of medians progressively decreased with the amount of RNA used. The sensitivity of hybridisation with viral sequences showed that approximately 0.5 μg total RNA from APLV-infected plants is sufficient for virus detection (Fig. 3b). However, this detection level might not apply to other viruses, or to the same virus in a different host plant.

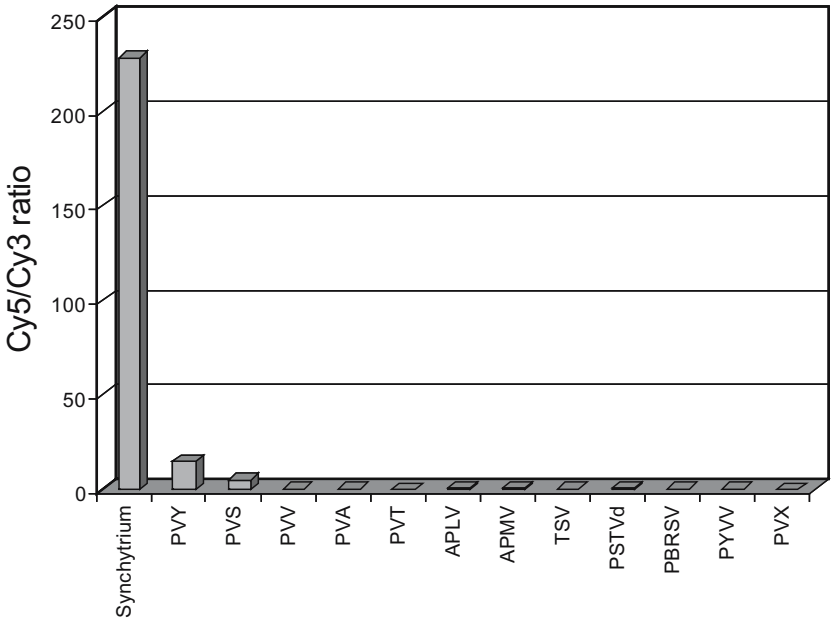

Fig. 2 Specificity of *Synchytrium*-specific oligonucleotide probes in microarray analysis. Total RNA from *Synchytrium*-infected potato tuber was labelled with Cy5 while RNA from healthy control plants was labelled with Cy3. The chart plots the ratio (Cy5/Cy3) of median pixel intensities (background subtracted) of fluorescence after hybridising the microarray. PVY *Potato virus Y*, PVS *Potato virus S*, PVV *Potato virus V*, PVA *Potato virus A*, PVT *Potato virus T*, APLV *Andean potato latent virus*, APMV Andean potato mottle virus, TSV *Tobacco steak virus*, PSTVd *Potato spindle tuber viroid*, PBRSV *Potato black ringspot virus*, PYVV *Potato yellow vein virus*, PVX *Potato virus X*

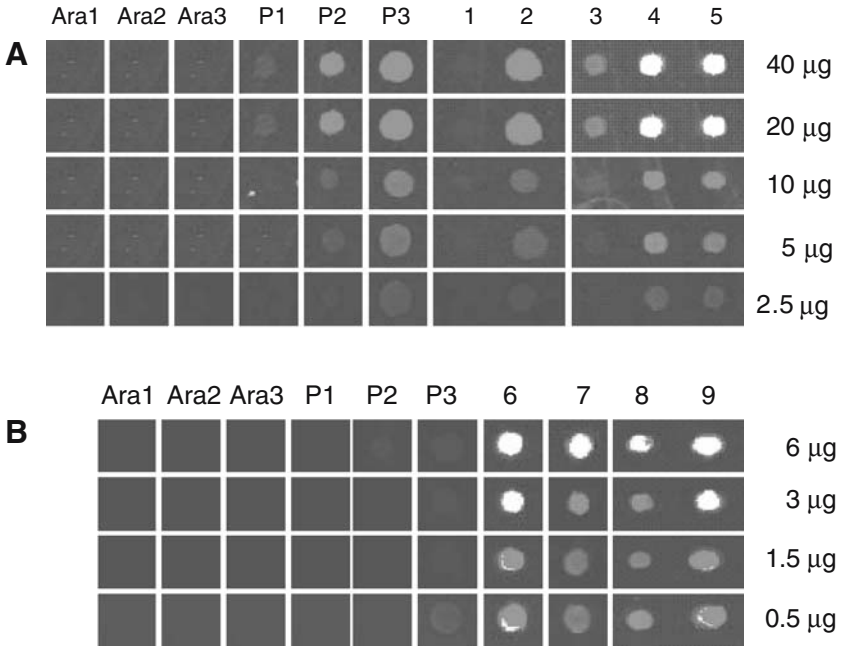

Fig. 3a, b Signal intensity variation with different amounts of labelled total RNA. **a** Approximately 2.5 µg, 5 µg, 10 µg, 20 µg and 40 µg RNA from wart-infected potato tubers was labelled separately and hybridised to four separate but similar microarrays. *Ara1*, *Ara2*, *Ara3* Oligonucleotides specific for *Arabidopsis thaliana* genes (negative controls); *P1*, *P2*, *P3* oligonucleotides representing NADH-ubiquitous oxidase, mitochondrial ATPase and 18S rRNA of potato/tobacco, respectively (positive controls); *1–5* probes a120boon091#1, a120boon093#1, a120boon090#1, a120boon092#1, a120boon094#1, respectively, designed to hybridise specifically with 18S rDNA of *Synchytrium endobioticum*. **b** Approximately 0.5 µg, 1.5 µg, 3.0 µg and 6.0 µg RNA from APLV-infected tobacco plants were treated as in **a**. *6–9* Probes a110boonham10#1, a120boon042#1, a120boon041#1, a120boon043#1, respectively, specific for APLV

Pixel intensity variations

The observed pixel intensities ranged from around background to saturation. This variation might also reflect the degree of complementarity of the probe to its target. To address this issue, the sequences of the oligonucleotide probes were compared with the target sequences (Table 1). No significant similarities were found between all five *Synchytrium*-specific probes and viral sequences. Despite this, probe 110boon093#1 produced a relatively high hybridisation signal with most of the viruses. This unspecific signal might result from hybridisation with a host plant gene. To investigate this, the 18S rDNA sequence of potato (>98% similarity to 18S rDNA of other solanaceous plants) was also aligned, and no significant similarity was observed with the five probes, except a110boon093#1, which showed 97% similarity (*e*-value=3e−10). This probably explains the observed high hybridisation signal with cDNA transcripts from healthy plants. The five *Synchytrium*-specific probes produced different signal intensities with

Table 1 Signal intensities of *Synchytrium*-specific oligonucleotide probes after hybridisation with different pathogens. PVA *Potato virus A*, PVS *Potato virus S*, PVM *Potato virus M*, PVY *Potato virus Y*, APLV *Andean potato latent virus*, WPMV *Wild potato mosaic virus*, PSTVd *Potato spindle tuber viroid*

Probe target	a120boon090#1		a120boon091#1		a120boon092#1		a120boon093#1		a120boon094#1	
	E-value[a]	Ratio[b]	*E*-value	Ratio	*E*-value	Ratio	*E*-value	Ratio	*E*-value	Ratio
PVA	ns[c]	1.3986	ns	0.0432	ns	−0.2542	ns	2.0251	ns	2
PVS	ns	1.2092	ns	−0.5043	ns	0.1572	ns	0.6341	ns	1.3524
PVM	ns	0.1662	ns	0.2089	ns	1.2245	ns	−0.4365	ns	−0.8329
PVY	ns	3.5142	ns	1.3943	ns	0.1827	ns	0.1617	ns	0.5386
APLV	ns	0.8236	ns	−0.5916	ns	0.9982	ns	2.0132	ns	0.3178
WPMV	ns	−0.6239	ns	0.9575	ns	0.4415	ns	1.8062	ns	−0.6493
PSTVd	ns	2.1621	ns	−0.3274	ns	1.2657	ns	−0.9928	ns	0.5739
Synchytrium	1e−16	1.0234	1e−16	0.0795	1e−16	247	1e−16	3.7826	1e−16	231

[a] *E*-values of the BLASTn search, used here as a measure of complementarity between target and probe, are compared with the ratio of pixel median intensities (minus background)
[b] Ratio (Cy5/Cy3) of Median pixel intensities (minus background) of fluorescence after hybridising microarray was calculated
[c] No significant similarity

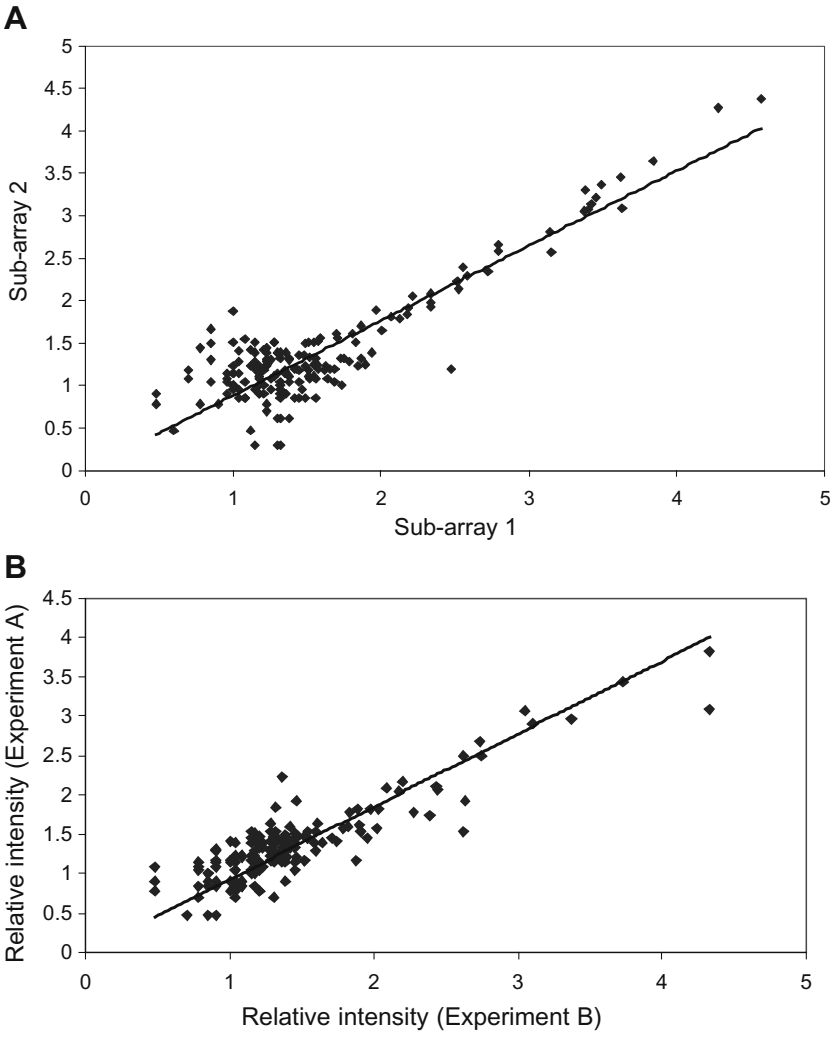

Fig. 4 **a** Reproducibility of fluorescence intensities of replicate oligonucleotide probes within an array. Total RNA from *S. endobioticum*-infected potato tuber was labelled with Cy5, and labelled cDNA was hybridised to the microarray. The logarithm of normalised median pixel intensities (minus background) of probes in the original sub-array (first replicate probes) was plotted against the intensities of the probes in the second sub-array. r=0.79. **b** Experiment-to-experiment reproducibility of fluorescent intensities. The logarithm of normalised median pixel intensity (minus background) from a microarray hybridised with *S. endobioticum* target in one experiment (experiment A) was plotted against the intensities for the same spots (probes) with another *S. endobioticum* target (experiment B). All conditions for the two experiments were the same. r=0.73

their target, while a110boon093#1 and a110boon091#1 did not yield positive hybridisation signals.

Reproducibility of results

To limit the variability that might result from RNA quality, aliquots of total RNA from a single extraction were used for repeated experiments. Visual inspection of pixel intensities generated from separate experiments revealed a generally high reproducibility, but oligonucleotides with outlier intensities were occasionally observed; such substantially different intensities were excluded from further analysis. To determine within-slide reproducibility, pixel signal intensities (median intensity minus background) of replicate spots (sub-arrays) were compared (Fig. 4a). The signal intensities of sub-array 1 were plotted against sub-array 2, and the relationship showed a high degree of correlation (r=0.79). Values from replicate spots within a slide were averaged, values derived from independent experiments were plotted, and regression analysis performed to assess array reproducibility (Fig. 4b). A correlation coefficient (r) of 0.73 was obtained, indicating high reproducibility of hybridisation between independent but similar experiments.

Discussion

We have demonstrated the potential of oligonucleotide-based microarray analysis for the detection of a range of plant pathogens. The availability of sequence information on pathogens, especially for viruses, has made the application of nucleotide-based technology easier. For *Synchytrium endobioticum*, however, no sequence information had previously been reported, hence our decision to sequence the ribosomal 18S DNA. Ribosomal 18S DNA is highly conserved among species, making possible the use of general primers such as NS1 and NS6 for PCR amplification. Despite such conservation, it was possible to design specific probes (a120boon092#1 and a120boon094#1) from the 1,421 bp 18S rDNA sequence, which consistently detected the fungus in the presence of other pathogens. *S. endobioticum* attacks only the underground parts of potato plants, thus microarray analysis using above-ground tis-

sues of infected plants indicated no infection. The four pathotypes tested in this study all produced similar levels of hybridisation signal; nonetheless, this does not mean that they are homogenous.

In the analysis of microarray results, hybridisation signals may be assessed visually to identify spots that produce fluorescence that is significantly above background. However, with larger amounts of microarray data it becomes increasingly difficult to analyse data on a visual basis. Moreover, signal intensity is a continuous range and it is impossible to establish a cut-off limit, hence the need for specialised software for data analysis. Before proceeding with the analysis, data were normalised to minimise the effect of unavoidable experimental variations. So far, a universally acceptable threshold value, or ratio of signal intensity, has not been established. For interpretation of our microarray data, we used the ratio of median signal intensity (minus background) of infected plants to healthy controls to determine positive hybridisation.

The results of hybridisation experiments repeated under the same conditions were significantly similar (r=0.73), and sub-arrays within slides also showed good correlation (r=0.79). The variations observed might have been due to several factors, ranging from sample preparation to image analysis, thus normalisation was applied. Our experiments revealed that not all oligonucleotides are equally useful in specifically detecting their target organisms. Sequence alignment of the probes and target organisms provided an indication for why false signals may be observed as a result of unspecific hybridisation between probes and host plant genes. Moreover, no correlation was observed between sequence complementarity and pixel fluorescence intensity (Table 1). Almost all viral gene-specific probes hybridised specifically to their target. In related studies, oligonucleotide assessment showed that, to reveal specificity, the gene-specific probe must be <75% similar to all non-target sequences to prevent significant cross-hybridisation (Kane et al. 2000; Boonham et al. 2003).

Besides specificity, sensitivity is another important factor to be considered, especially when the quantity of available RNA is limiting. Hybridisation signal intensity for oligonucleotides perfectly complementary to their targets varied from none to very high. There is convincing evidence that base composition is not the main determinant of the hybridisation reaction. The formation of secondary structures by the target sequence, a major determinant affecting its interaction with other molecules, affects availability for hybridisation (Sohail et al. 1999). In this study, we showed that ~2.5 μg and 0.5 μg total RNA are sufficient for detection of *S. endobioticum* and APLV, respectively. However, this sensitivity level is not the same for other pathogens. Unless a limited amount of RNA is available, we recommend the use of a greater quantity to ensure that a signal significantly above background is obtained.

Synthesised oligonucleotides proved to be a convenient method of microarray production, and have the added advantage that probes can be designed to detect multiple variant regions of a transcript. Furthermore, by selecting microarray elements derived from highly conserved regions within families, individual pathogens that were not explicitly represented on the microarray could be detected, raising the possibility that all members of each family, including unsequenced, unidentified, or newly evolved family members may well be detected. Some of the shortfalls observed in this microarray could be improved by better probe selection, addition of more probes and deletion of non- or cross-hybridising probes. In summary, this study suggests that oligonucleotide-based microarray has great potential, providing a very convenient option for multiple pathogen detection, especially in quarantine laboratories where plant materials are routinely screened for both characterised and uncharacterised disease-causing organisms.

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