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Physical mapping across an avirulence locus of *Phytophthora infestans* using a highly representative, large-insert bacterial artificial chromosome library

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Abstract The oomycete plant pathogen *Phytophthora infestans* is the causal agent of late blight, one of the most devastating diseases of potato worldwide. As part of efforts to clone avirulence (*Avr*) genes and pathogenicity factors from *P. infestans*, we have constructed a bacterial artificial chromosome (BAC) library from an isolate containing six *Avr* genes. The BAC library comprises clones with an average insert size of 98 kb and represents an estimated 10 genome equivalents. A three-dimensional pooling strategy was developed to screen the BAC library for amplified fragment length polymorphism (AFLP) markers, as this type of marker has been extensively used in construction of a *P. infestans* genetic map. Multiple positive clones were identified for each AFLP marker tested. The pools were used to construct a contig of 11 BAC clones in a region of the *P. infestans* genome containing a cluster of three avirulence genes. The BAC contig is predicted to encompass the *Avr11* locus but mapping of the BAC ends will be required to determine if the *Avr3* and *Avr10* loci are also present in the BAC contig. These results are an important step towards the positional cloning of avirulence genes from *P. infestans*, and the BAC library represents a valuable resource for large-scale studies of oomycete genome organisation and gene content.

Keywords Plant pathogen · Genomics · Oomycete · Tomato

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Introduction

Phytophthora infestans is the causal agent of late blight of potato and tomato, and is responsible for significant losses of these crops worldwide (Erwin and Ribeiro 1996). This is particularly the case for the cultivated potato, where the organism is regarded as a threat to global food security. *P. infestans* belongs to the oomycetes, a class of organisms that includes many important plant pathogens. The oomycetes exhibit a mycelial growth habit but are distinct from the true fungi (Hawksworth et al. 1995; Erwin and Ribeiro 1996). *P. infestans* exhibits a gene-for-gene interaction with potato, in which avirulence gene (*Avr*) products from the pathogen are recognised by the host plant expressing the cognate resistance gene (van der Lee et al. 2001). This leads to the hypersensitive response (HR) – localised programmed cell death – in the plant that inhibits further spread of the pathogen.

At the molecular level, *P. infestans* is becoming a model oomycete for the study of pathogenicity mechanisms and avirulence. Central to these studies are resources such as a molecular-genetic linkage map (van der Lee et al. 1997), an expressed sequence tag (EST) database (Kamoun et al. 1999), as well as techniques for the analysis of gene function, such as transformation (reviewed in Judelson 1997), gene silencing (van West et al. 1999), *in planta* reporter systems (Kamoun et al. 1998) and heterologous expression of genes from other *Phytophthora* species (Panabieres et al. 1998). DNA libraries, particularly large-insert genomic libraries, are a critical resource for structural genomics and, by definition, for positional cloning.

The system most amenable to the manipulation of large cloned DNA inserts is the *Escherichia coli* bacterial

artificial chromosome (BAC; Shizuya et al. 1992). These cloning vectors, based on a single-copy F factor plasmid, are capable of stable maintenance of insert DNA of up to 300 kb in length. Insert sizes above 100 kb are preferred for positional cloning applications, since fewer clones are required for chromosome walking across, or chromosome landing within, linkage map intervals containing genes of interest. This is of particular importance if the genome concerned is large, or if the gene of interest lies in a genomic region where the frequency of recombination is low. The genomes of many fungal and oomycete plant pathogens are relatively small compared to those of other organisms in which positional cloning has been undertaken (Voglmayr and Greilhuber 1998). However, the genome size of *P. infestans* has been estimated to be 250 Mb (Tooley and Therrien 1987), which is relatively large for an oomycete plant pathogen, and larger than the genome of the model plant *Arabidopsis thaliana*.

When producing a BAC library that is to be used primarily for the positional cloning of multiple target genes, it is more efficient to use a heterozygous individual from a selected F_1 mapping population as a source of high-molecular-weight DNA. An F_1 from a mapping population that possesses all required markers and phenotypic traits can then be selected if all the target genes segregate in the population. Recently, using a segregating population, the positions of six dominant *Avr* genes have been located on the molecular genetic linkage map of *P. infestans*. All have tightly linked AFLP markers (van der Lee et al. 2001). Using one F_1 from this population, which contains all six *Avr* genes from the genetic cross described by van der Lee et al. (1997, 2001), we set out to (1) produce a BAC library representing a ten-fold coverage of the genome with an average insert size of 100 kb, and (2) construct a contig of BAC clones across one specific region of the *P. infestans* genome that contains a large number of AFLP markers and a cluster of *Avr* genes. This BAC library will be a valuable resource for positional cloning and for large-scale studies of oomycete genome organisation and gene content. Chromosome landing near an avirulence locus is an important step towards the cloning and characterisation of these genes, which are of crucial importance in the interaction of potato with *P. infestans*.

Materials and methods

P. infestans isolates

The F_1 individual T30-4, obtained from the genetic cross used to construct the linkage map of *P. infestans* (van der Lee et al. 1997), contains all six avirulence genes (*Avr1, Avr2, Avr3, Avr4, Avr10* and *Avr11*) segregating in the cross, and was used as a source of high-molecular-weight DNA for construction of the BAC library. The parental isolates 80029 (race 2.4.7; A1 mating type) and 88133 (race 1.3.7.10.11; A2 mating type) were used for verification of AFLP alleles present in the BAC library. Cultures were maintained on rye agar plates (Ribiero 1978) at 18°C. Sporangia and mycelial fragments, scraped from the agar plate cultures, were used to inoculate pea broth for large-scale preparation of mycelium.

Preparation of high-molecular-weight DNA from *P. infestans*

Three day old pea-broth cultures of T30-4 were filtered through Miracloth (Calbiochem) and the young mycelium protoplasted as described by Judelson et al. (1993). Typically, *P. infestans* mycelium was completely digested into protoplasts after 45 min at 25°C. Protoplasts were harvested, washed, embedded in agarose, and lysed as described by Judelson et al. (1993). Agarose plugs containing intact chromosomal DNA were then washed twice in 0.5 M EDTA pH 8.0 at 4°C, twice in 50 mM EDTA pH 8.0 at 4°C, and five times in TE buffer (10 mM TRIS-HCl, 1 mM EDTA) pH 8.0 at 4°C. Plugs were stored at 4°C in TE buffer prior to use.

Partial restriction digestion of high-molecular-weight DNA

Optimal conditions for partial digestion of the high-molecular-weight DNA were determined by titrating the restriction endonuclease (*Hin*dIII or *Bam*HI) against the DNA. An agarose plug containing high-molecular-weight DNA was reduced to a fine slurry with a razor blade on a microscope slide. This slurry was resuspended in 1 ml of TE buffer and 4 µl of 25% Triton X-100, collected by brief low-speed centrifugation in a microcentrifuge, and excess TE buffer was removed. Aliquots of 50 µl were transferred to microcentrifuge tubes and equilibrated on ice for 1 h with the appropriate restriction endonuclease buffer, BSA (100 µg/ml), and spermidine (4 mM). Enzyme dilutions in 5 µl (1 U, 0.5 U, 0.25 U, 0.125 U, 0.06 U, and 0 U) were added to each tube and allowed to equilibrate on ice for 30 min. Restriction digests were then carried out at 37°C for 30 min, stopped by addition of EDTA to 50 mM, and kept on ice until loaded on a CHEF gel. Restriction fragments were separated on a 1% agarose gel in 1×TAE buffer, using a CHEF Mapper (Bio Rad) with a constant 20-s pulse time, an 18-h run time at 6 V/cm, a 120° included angle, and a constant temperature of 13°C. After staining in ethidium bromide, the optimal digest conditions were identified as those yielding the greatest amount of DNA in the range of 100–250 kb. For large-scale digestion, the conditions determined for the partial digestion were scaled up, except that the reaction volumes were kept at 70 µl, so as to reproduce as closely as possible the optimal digestion conditions. Restriction fragments were separated as described above. DNA was recovered from the gel by electroelution (5 V/cm, 4°C, 1×TAE, 3 h) from gel fragments punched out of the size range 130–250 kb using Quik-Pik electroelution capsules (Stratagene). Alternatively, DNA was electrophoresed on a second CHEF gel as follows. The section of the gel containing the partially digested DNA between 120–250 kb was excised and transferred to the top of a new 1% agarose CHEF gel, but in the reverse orientation such that the smallest fragments were located at the top of the gel. Electrophoresis was then performed using the same CHEF conditions as previously. This had the effect of concentrating partially digested DNA into a single band which was easier to recover, at a higher concentration, using electroelution. TAE buffer was removed by drop dialysis on 0.2 µm membranes (Millipore) against TE buffer at 4°C for at least 3 h. Concentration of the DNA samples, if required, was also performed by drop dialysis against 0.5×TE buffer containing 30% polyethylene glycol 8000 (Sigma).

Preparation of the pBeloBAC11 vector

The BAC vector pBeloBAC11 (Kim et al. 1996) was prepared on a large scale from a 5 l LB broth culture containing 12.5 µg/ml chloramphenicol, which was grown for 24 h before harvesting. DNA was isolated using the Qiagen Giga Prep procedure for very low-copy number plasmids. Contaminating *E. coli* genomic DNA was degraded using Plasmid Safe exonuclease (Epicentre Technologies). pBeloBAC11 was digested with a ten-fold excess of either

*Hin*dIII or *Bam*HI. Dephosphorylation with shrimp alkaline phosphatase (Roche) was carried out at the same time as the restriction digest.

Ligation of *P. infestans* DNA into pBeloBAC11

For optimal ligation of partially digested DNA into BAC vectors, the conditions determined by Osoegawa et al. (1998) were used: a vector:insert ratio of 10:1, and final concentrations of 0.5 and 1 ng/μl of vector and insert DNA, respectively. Digested, dephosphorylated vector (10 ng) was tested for self-ligation. Ligations were carried out for 14–16 h at 14°C.

Electroporation of *E. coli* DH10B cells

A 1 μl aliquot of the ligation reaction was mixed with 20 μl of Electromax DH10B cells (Gibco-BRL) and electroporated at 1.8 kV (BioRad *E. coli* Gene Pulser). Then 1 ml of SOC medium was mixed with the transformed cells and incubated at 37°C for 1 h before plating onto LB agar containing 12.5 μg/ml chloramphenicol, 100 μg/ml X-gal, and 100 μg/ml IPTG. Plates were incubated for 24 h at 37°C, and then transferred to 25°C in darkness for a further 24 h to allow for development of blue/white colonies.

Determination of average insert size and arraying of BAC clones

The average insert size in 30–40 recombinant clones from a single ligation was determined prior to arraying of clones for long-term storage as part of the BAC library. BAC DNA was prepared by alkaline lysis of 4-ml 24-h cultures of white colonies from X-gal agar plates. Isolated BAC DNA was digested with *Not*I restriction endonuclease, and loaded onto a 1% agarose CHEF gel in 1×TAE buffer. Restriction fragments were separated under the following conditions: a constant temperature of 13°C, a 5–15 s switch time with linear ramping, a voltage gradient of 6 V/cm, 120° included angle, and a run time of 18 h. Ligations that exhibited an average insert size over 80 kb were used for library construction. BAC clones were picked individually into 384 well microtitre plates containing LB-freeze medium (LB broth + 36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM sodium citrate, 400 μM $MgSO_4$, 6.8 mM $(NH_4)_2SO_4$, 4.4% glycerol) with 12.5 μg/ml chloramphenicol. Inoculated microtitre plates were grown for 24 h at 37°C, replicated twice, and then frozen at –80°C.

High-density filters of BAC clones

Clones from the BAC library were transferred to Hybond N+ nylon membranes (Amersham Pharmacia Biotech) using a Beckman Biomek 2000 Laboratory Workstation. Clones from eight 384 well plates were replicate-transferred to a membrane of 7.8 cm×11.9 cm in a 4×4 array, so that each filter contained 3072 BAC clones. Each clone was double spotted in a unique pattern and the position of the clones in the array was designed to be diagnostic of the plate from which the clones originated. The entire BAC library was thus represented on nine filters. Clones arrayed on membranes were grown for 24 h at 37°C on LB-chloramphenicol agar before the bacterial colonies were lysed with alkali to bind the DNA to the membrane.

Probe labelling and DNA hybridisation

High-density filters were screened by hybridisation with probes derived from four genes that are known to be single copy genes in *P. infestans*: *calA* encoding calmodulin (Pieterse et al. 1993), *niaA* encoding nitrate reductase (Pieterse et al. 1995), *actB* encoding actin (Unkles et al. 1991), and the *in planta* induced gene IPIDI (Pieterse et al. 1994). Filters were also hybridised with the internal transcribed spacer (ITS) region of the rDNA repeat from *P. infestans* (Cooke et al. 2000). Probes (25 ng) were labelled by random priming using the High Prime labelling system (Roche). Prehybridisation and hybridisation were carried out at 65°C in a solution containing 7% SDS in 0.5 M sodium phosphate buffer pH 7.2, and 1 mM EDTA. After hybridisation, membranes were washed twice in 5×SSPE buffer (Sambrook et al. 1989) at 65°C for 20 min each, and twice in 1×SSPE + 0.1% SDS at 65°C for 20 min each. After exposure to Biomax MS X-ray film (Kodak), membranes were stripped for reuse as described by Braithwaite et al. (1991).

Pooling of BAC clones

To facilitate screening of the BAC library by PCR-based methods, pools of BAC clones were constructed in "three dimensions". First, the library was separated into odd and even numbered plates, with 36 plates in each group. Row (A–P) and column (1–24) pools were constructed for each group of plates. One odd numbered plate and one even numbered plate were pooled to form layer pools. Each layer pool contained 768 BAC clones, each row pool contained 864 BAC clones, and each column pool contained 576 BAC clones. Pooled BAC clones were grown at 37°C on LB-chloramphenicol agar. Cells were harvested by scraping colonies from the agar surface in 15 ml of LB broth. BAC DNA from the pooled clones was isolated by alkaline lysis.

AFLP DNA fingerprinting

AFLP DNA fingerprinting was performed essentially as described previously (Vos et al. 1995) but with some minor modifications. BAC DNA (50 ng) was digested with *Eco*RI/*Mse*I, and preamplified with non-selective primers. Preamplified DNA was diluted tenfold in water before amplification with selective primers. After the selective PCR with E+2 and M+2 primers, the liquid in the samples was not evaporated prior to the addition of formamide dye (98% formamide, 20 mM EDTA, with xylene cyanol and bromophenol blue as tracking dyes).

Results and Discussion

Construction of a *P. infestans* BAC library

When constructing BAC libraries, difficulty is generally encountered in routinely obtaining clones with average insert sizes of over 100 kb, despite the fact that the DNA fragments selected for cloning are in the correct size range (Diaz-Perez et al. 1996; Osoegawa et al. 1998; Randall and Judelson 1999). We also experienced this difficulty. Initial attempts to use a single size selection of partially *Hin*dIII-digested *P. infestans* DNA for construction of the BAC library predominantly yielded inserts averaging less than 70 kb. However, by selecting partially *Hin*dIII-digested DNA from the size range of 130–250 kb, BAC inserts with an average insert size of 89 kb were obtained (ligation A, Table 1). However, the variation in insert size was large (25–225 kb). Clones estimated to represent 2.7 genome equivalents were picked into 384-well microtitre plates. It was surmised that the smaller DNA fragments were being trapped in the section of the CHEF gel containing the larger restriction fragments, and this was leading to a reduction in the average insert size. Others have used methods such as initial reversal of the polarity of electrophoresis to remove small fragments from the CHEF gel, followed by separation of larger restriction fragments using typical

Table 1 Average insert sizes and genome representation obtained from the five ligations used in construction of the *P. infestans* BAC library

Ligation	Plates	Cloning enzyme	Average insert size (kb)	Genome representation
A	1–20	*Hin*dIII	89	2.7×
B	21–30	*Hin*dIII	96.5	1.0×
C	31–33	*Hin*dIII	100	0.5×
D	34–40	*Hin*dIII	98.5	1.1×
E	41–53	*Bam*HI	112	2.2×
D	54–72	*Hin*dIII	98.5	2.7×

CHEF gel conditions (Osoegawa et al. 1998). We adopted an alternative approach of two size selections to enrich the DNA preparation for larger fragments. The section of the CHEF gel containing the desired size range of fragments was excised, inverted, and electrophoretically fractionated in a second CHEF gel under the same conditions. This had the effect of concentrating the larger DNA fragments into a single band, while the small fragments, which previously co-migrated with larger fragments, were observed as a faint smear below the major band of DNA after staining with ethidium bromide. Ligations using DNA prepared by this method yielded average insert sizes ranging from 97 kb to 100 kb (ligations B–D, Table 1). Some small inserts were still observed in sections of the BAC library prepared in this way. However, they were less numerous and the average insert size was close to, or exceeded, 100 kb. The remaining targeted 7.3-fold genome coverage of the BAC library was established using DNA prepared in this manner.

To avoid bias in genomic representation, two separate restriction endonucleases were used in constructing the BAC library; *Hin*dIII, which has a recognition sequence that is AT-rich, and *Bam*HI, which has a GC-rich recognition sequence. Clones containing an estimated 2.2 genome equivalents were picked from one ligation of DNA prepared using *Bam*HI as the restriction enzyme (ligation E; Table 1). The average insert size (112 kb) for this section of the BAC library was greater than that from any of the *Hin*dIII ligation reactions, and the size range was from 80–170 kb.

The complete BAC library, arrayed in 72×384 well microtitre plates, comprises an estimated 10.2 genome equivalents with an average cloned insert size of 98 kb. If ligation A (2.7 genomes, 89 kb average insert size) is excluded from calculations, the average insert size for the *P. infestans* BAC library would be 102 kb.z

Assessing genomic representation in the BAC library by hybridisation

To ascertain the representation of single copy sequences within the library, high-density filters containing DNA from the BAC clones were screened by hybridisation with probes derived from four genes that are known to be single copy genes in *P. infestans*. Using these genes as probes, four BAC clones containing the *niaA* gene were identified, five for *ipiO*, seven for *actB*, and eight for *calA*.

To determine the representation of ribosomal DNA in the BAC library, filters were hybridised with the internal transcribed spacer region (ITS) from *P. infestans* (Cooke et al. 2000). It was estimated that ribosomal DNA is present in at least 2.3% of all clones. Interestingly, no BAC clones containing the ITS region were observed in the portion of the BAC library generated using *Bam*HI as a cloning enzyme.

PCR-amplified fragments of three *P. infestans* mitochondrial genes (*cox1, ATP1, nad11*; GenBank Accession No. U17009; Paquin et al. 1997) were pooled and used as a probe. No BAC clones were identified which hybridised to this mixture of probes, demonstrating that the size selections of partially digested DNA were successful in excluding the small (38-kb) mitochondrial DNA molecule.

Construction of a BAC contig spanning an avirulence locus on Linkage Group VIII

The BAC library was constructed to facilitate positional cloning of *Avr* genes. Recently, Randall and Judelson (1999) described the construction of a *P. infestans* BAC library that comprises clones with an average insert size of 75 kb, representing 4.9 genome equivalents. However, the *P. infestans* isolate used by Randall and Judelson (1999) for BAC library construction has a different genetic background to that of the F_1 individual used here, and may not contain the same *Avr* gene-linked AFLP markers.

Pools of BAC clones were developed to allow screening with AFLP markers linked to *Avr* genes. The genomic region selected for BAC contig construction was a dense cluster of AFLP markers positioned at one end of Linkage Group VIII (Fig. 1). Three avirulence genes (*Avr3*, *Avr10* and *Avr11*) have been genetically mapped to this region (van der Lee et al. 2001). The three-dimensional pools of BAC clones were initially screened with eight AFLP primer combinations that amplify fragments that map to this region (indicated in bold in Fig. 1). Due to the structure of the BAC pools and the identification of multiple positive clones, n^3 potential clone addresses (where n is the number of positive BAC pools) require a second course of AFLP screening to identify the genuine positives. For example, where five positive clones were identified in one half of the BAC library, 125 potential clones needed to be rescreened. Individual BAC clones from potential clone addresses, identified from positive BAC pools, were

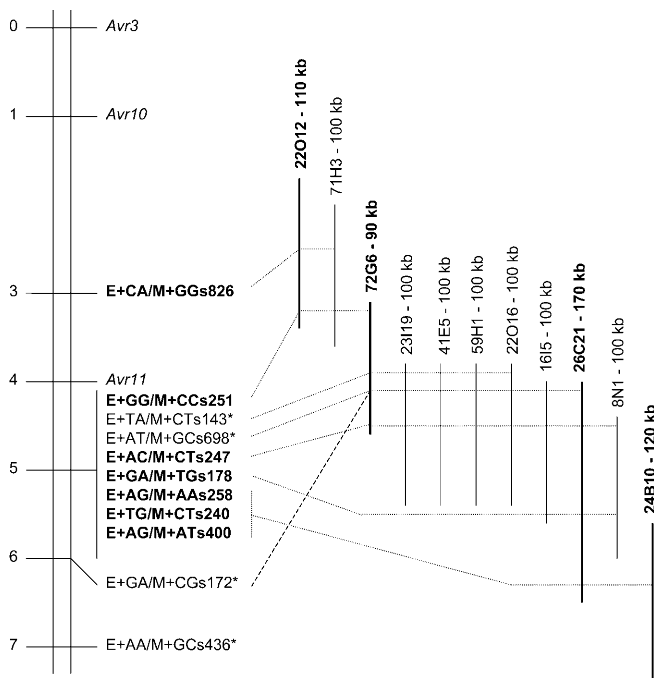

Fig. 1 Section of linkage group VIII of *P. infestans* (*left*) (adapted from van der Lee et al. 2001). Indicated are the map positions of AFLP markers used in the first (*bold type*) and second (*asterisks*) screens used to identify and verify BAC clones spanning this genomic region. BAC clones that could be assembled into contigs (not to scale) are indicated as *solid vertical lines* to the *right* of the linkage group map. Their respective insert sizes are indicated at the *top* of the solid vertical lines. BAC clones shown in *bold* represent the minimum tiling path across the region. The relative positions of AFLP markers in the BAC contig are shown by *dotted lines*. The position in the BAC contig of AFLP marker E + GA/M + CGs172, which was mapped outside the major cluster of AFLP markers, is indicated by the *dashed line*

re-screened by AFLP fingerprinting to ascertain the addresses of the actual positive BAC clones.

Since dominant PCR-based markers are represented in the BAC library as alleles, it was expected that approximately five positive BAC clones would be identified per polymorphic AFLP marker. Between two and eight positive BAC clones per AFLP marker were identified after the secondary screen. The number of positive BAC clones for AFLP markers is consistent with the calculated genome redundancy in the BAC library, and therefore also consistent with the estimated genome size for *P. infestans*.

Positive BAC clones assembled into contigs, and their corresponding insert sizes, are shown in Fig. 1. From this screen, four BAC clones were observed to contain more than one AFLP marker. Clone 26C21 was observed to contain five of the markers screened. BAC clones were further analysed for specific AFLP fragments using four additional primer combinations from the cluster of markers at the end of LG VIII. This further confirmed the preliminary contig of BAC clones and joined BACs 72G6, 71H3, and 22O12 to the larger part of the contig. By linkage mapping, marker E + GA/M + CGs172 is located at 6 cM on the linkage map. However, this marker was determined to be physically located within the BAC contig, amplifying from the same BAC clones as E + AT/M + GCs698. This situation may have arisen if one F_1 individual in the mapping population had been mis-scored for this marker. In contrast, marker E + AA/M + GCs436 could not be amplified from any of the BAC clones in the contig, demonstrating that the contig does not extend as far as this marker.

BAC clones were digested with restriction enzymes, individually and in combination, to determine the overlap between clones and form contigs spanning the genomic region. By this approach, it was determined that all but two of the identified BAC clones formed a single contig (Fig. 1). Both of the unassembled clones were identified using the EAA + /M + CTs239 marker. Since the AFLP markers in this region are densely clustered, and therefore difficult to order accurately, it is likely that these two BAC clones lie further away from the clones in the contig. This could be confirmed by testing more markers in this region, or mapping the ends of these BAC clones.

Although the contig contains 11 BAC clones, the minimum tiling path across the region is represented by only four clones: 22O12, 72G6, 26C21, and 24B10 (Fig. 1). Given the size of these clones and the clear overlap between them, as revealed by both AFLP and restriction digestion analyses, the minimum contig contains less than 490 kb of DNA (the sum of BAC inserts in the minimum tiling path).

The genetic position of *Avr11*, as determined by van der Lee et al. (2001) suggests that this gene should be contained within the BAC contig. Mapping of the ends of the BAC contig on the AFLP linkage map should reveal whether or not the BAC contig also contains *Avr3* and *Avr10*. If *Avr*3 and *Avr*10 cannot be demonstrated to lie within the BAC contig, then a chromosome walk will be required to extend the BAC contig to encompass these loci.

Concluding remarks

In this report we have presented a physical map of an avirulence locus in *P. infestans*, and this represents an important step towards the positional cloning of the first *P. infestans Avr* gene. Recently, Randall and Judelson (1999) reported successful transformation and stable integration of entire BACs into the genome of *P. infestans*. This approach could be used to confirm the presence of *Avr* genes within the BAC contig presented here. Transformation of entire BACs containing markers flanking *Avr* loci into *P. infestans* isolates lacking the *Avr* allele should result in a change in phenotype from virulent to avirulent on a potato line carrying the corresponding resistance gene. In this instance, the four BAC clones forming the minimum tiling path across the region would be used to determine which, if any, contain

Avr genes. Subcloning of the BACs, followed by further transformation experiments could then be used to identify the fragment of genomic DNA containing an *Avr* gene. Alternatively, the entire BAC contig could be sequenced, ORFs predicted, and each ORF tested by transformation for function as an *Avr* gene.

Further to positional cloning of genes, the BAC library will also serve as a valuable resource for future genomic studies of *P. infestans* and other plant pathogenic oomycetes. For *P. infestans*, the existence of a genetic linkage map (van der Lee et al. 1997) and a set of expressed sequence tags (ESTs) (Kamoun et al. 1999) would make it possible to integrate transcriptional, physical and genetic maps. Moreover, the BAC library will underpin efforts directed at understanding genome structure in *P. infestans* through comparisons with genome structure in other oomycetes, such as the soybean pathogen *P. sojae* (Mao and Tyler 1991; Whisson et al. 1995) and *Peronospora parasitica*, a pathogen of the model plant *Arabidopsis thaliana* (Voglmayr and Greilhuber 1998; Rehmany et al. 2000). Such efforts will lead to an enhanced understanding of these important plant pathogens and support the development of novel strategies for their control.

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