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Construction of a YAC library from a *Beta vulgaris* fragment addition and isolation of a major satellite DNA cluster linked to the beet cyst nematode resistance locus *Hs1*$^{pat-1}$

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Abstract A YAC library was constructed from the *Beta vulgaris* fragment addition AN5-203b. This monosomic fragment addition harbors an approximate 12-Mbp fragment of *B.patellaris* chromosome 1 accomodating the *Hs1*$^{pat-1}$ locus conferring resistance to the beet cyst nematode (*Heterodera schachtii*). The YAC library consists of 20,000 YAC clones having an average size of 140 kb. Screening with organelle-specific probes showed that 12% of the clones contain chloroplast DNA while only 0.2% of the clones hybridizes with a mitochondrial specific probe. On the basis of a sugar beet haploid genome size of 750 Mbp this library represents 3.3 haploid genome equivalents. The addition fragment present in AN5-203b harbors a major satellite DNA cluster that is tightly linked to the *Hs1*$^{pat-1}$ locus. The cluster is located on a single 250-kb *Eco*RI restriction fragment and consists of an estimated 700–800 copies of a 159-bp core sequence, most of which are arranged in tandem. Using this core sequence as a probe, we were able to isolate 1 YAC clone from the library that contains the entire 250-kb satellite DNA cluster.

Key words Nematode resistance
Pulsed field electrophoresis · Satellite DNA · Sugar beet
Yeast artificial chromosome library

Abbreviations *YAC* Yeast artificial chromosome
BCN beet cyst nematode
RAPD random amplified polymorphic DNA
RFLP restriction fragment length polymorphism

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Introduction

The beet cyst nematode *Heterodera schachtii* Schm. is a severe pest in most areas of sugar beet (*Beta vulgaris* L.) cultivation and can cause considerable losses in yield annually. Since crop rotation cannot fully overcome the nematode problem and the use of nematicides increasingly forms a threat to the environment, the introduction of resistant sugar beet varieties is highly desirable. However, no useful level of resistance is present in *B.vulgaris*. Resistance genes against *H.schachtii* were identified in wild beets (*B. procumbens* Chr. Sm. and *B. patellaris* Moq.) belonging to the section *Procumbentes* (Coons 1975; Yu 1984). In *B. procumbens* two loci conferring resistance are present, *Hs1*$^{pro-1}$ on chromosome 1 and *Hs2*$^{pro-7}$ on chromosome 7. In *B. patellaris* the *Hs1*$^{pat-1}$ locus on chromosome 1 confers resistance against the nematode (nomenclature according to Lange et al 1993, for further references see Lange et al 1990; Van Geyt et al 1990). Attempts have been made to transfer the nematode resistance genes to *B. vulgaris* through interspecific crossings. While direct gene transfer appeared to be impossible, resistant monosomic additions (2 n = 18 + 1) containing *B. patellaris* chromosome 1, *B. procumbens* chromosome 1, or *B. procumbens* chromosome 7 were obtained. The backcrossing of resistant monosomic additions with diploid *B. vulgaris* resulted in resistant diploid cytotypes (Heijbroek et al 1988; Jung and Wricke 1987; Savitsky 1978). However, genetic studies suggest that the insertion in the recipient genome of the alien piece of chromosome carrying the gene for resistance is unstable (Lange et al 1990). The alien chromosomes in the monosomic additions also showed breakage, leading to resistant backcross individuals in which the resistance is located on a telosome (Speckmann et al 1985) or on a chromosome fragment (Brandes et al 1987; De Jong et al 1986) The chromosome fragments especially are poorly transmitted through meiosis, and they may also show some mitotic instability.

To obtain genetically stable nematode resistant sugar beet varieties, cloning of the resistance gene from one of

the wild beet species and the subsequent introduction of the gene in *B. vulgaris* forms a powerful alternative to the introduction of the resistance by classical plant breeding. Cloning of this gene, however, can not be carried out using the conventional molecular cloning strategies since no gene product from the BCN resistance gene is known. Instead, strategies such as map based cloning (Orkin 1986) can be followed in which molecular markers, like RFLP markers or RAPD markers, tightly linked to the gene of interest are identified. The markers are used to screen DNA libraries containing very large stretches of cloned DNA. These libraries mostly are constructed in YAC vectors (Burke et al 1987), which can accommodate insert DNA up to the megabase-size range (Chumakov et al 1992; McCormick et al 1989). By means of chromosome walking the isolated YAC clones are arranged into contigs spanning the region in which the gene is located. Subsequently, candidate genes are isolated from the YAC clones and the gene of interest is identified by functional analysis and/or complementation studies. The feasibility of map based cloning for the isolation of plant genes has been demonstrated by the successful isolation of a gene encoding an omega-3 desaturase (Arondel et al 1992), the ABI3 gene (Giraudat et al 1992) and the ETR1 gene (Chang et al 1993) all from *Arabidopsis thaliana* and, recently, by the cloning of the *Pto* gene from tomato conferring resistance against *Pseudomonas syringae* pv. tomato (Martin et al 1993).

The map-based cloning of other disease-resistance genes from various important crop species can be foreseen in the near future; molecular markers linked to genes acting against plant pathogenic bacteria (Ronald et al 1992; Kunkel et al 1993), fungi (Leonards-Schippers et al 1992; Sarvatti et al 1989; Schüller et al 1992), nematodes (Barone et al 1990; Jung et al 1992; Klein-Lankhorst et al 1991; Kreike et al 1994; Salentijn et al 1992; Weiseman et al 1992) and viruses (Ritter et al 1991; Young et al 1988) have been isolated and, in addition, the construction of YAC libraries of crop species such as maize, barley, carrot, and tomato have been described (Edwards et al 1992; Guzmán and Ecker 1988; Kleine et al 1993; Martin et al 1992). Also, the construction of a YAC library from sugar beet line Ar^+ has been reported recently (Eyers et al 1992) which, however, is a nematode-susceptible line. As a first step towards the map-based cloning of the beet cyst nematode resistance gene, we have constructed a YAC library from the resistant *B. vulgaris* fragment addition AN5-203b. This fragment addition contains a 12 Mbp chromosomal fragment derived from *B. patellaris* chromosome 1 on which the resistance gene is located. The library was screened with a wild beet-specific satellite DNA (Salentijn et al 1992), resulting in the isolation of a YAC clone containing a 250-kb satellite DNA cluster that is tightly linked to the BCN gene.

Materials and methods

Chemicals

Zymolyase-100T was obtained from Seikagaku Kogyo Co, Lyticase, amino acids, adenine hydrochloride, and sorbitol were from Sigma, and the type of glucose "for microbiology" was from Merck. All growth media were obtained from Difco. High-molecular-weight DNA markers were from BioRad. Restriction and modification enzymes and β-agarase were obtained from New England Biolabs. Proteinase K was purchased from Boehringer. All agarose types used were from FMC.

Strains and plasmids

Saccharomyces cerevisiae AB1380 (Burke et al 1987), pYAC4 (Burke et al 1987), and pPHcPs1 (Van Grinsven et al 1986) were obtained from R. van Daelen (LUW, Wageningen). Sat-121 (formerly referred to as 121.3) was isolated by Salentijn et al (1992). pCOXII (Fox and Leaver 1981) was obtained from C. Kick (CPRO-DLO, Wageningen), and p663 was a gift of C. Jung (Christian-Albrechts Universität, Kiel). The yeast growth and selection media were prepared according to Gibson and Somerville (1991).

Plant DNA isolation

Total DNA was isolated (Bernatzky and Tanksley 1986) from leaf tissue sampled from at least five individual plants. High-molecular-weight (HMW) DNA was isolated basically as described by Schwartz and Cantor (1984). Mesophyll protoplasts (pps) were isolated from young leaves (20–50 mm) taken from at least five individuals of plant material kept under greenhouse conditions. The pps were isolated according to a method described by Krens et al (1990) with the following modifications. No preplasmolysis was performed, and *n*-propyl-gallate was omitted from the enzyme mixtures (1% (w/v) cellulase R-10 and 1.5% (w/v) macerozyme R-10). After an incubation time of 3 h the pps were collected from the enzyme mixture and washed once with CPW-salts containing 9% mannitol. After isolation the pps were concentrated to a final concentration of 8×10^6 pps per 100 microliters by centrifugation (800 rpm), mixed (1 : 1) with 1.5% Incert agarose (FMC) in 0.25 M EDTA, and quickly poured into molds (V = 100 microliters). The final pps concentration in the agarose plugs of 4×10^6 pps is equivalent to 6.3 µg DNA (based on a DNA content of 1.57 picograms for the diploid *Beta* genome (Arumuganathan and Earl 1991). After solidification the plugs were immediately incubated twice for 24 h in 0.5 *M* EDTA supplemented with 1 mg/ml proteinase K and 0.02 *M* sodiumbisulphite (V = 15 ml per 8 plugs) at 50 °C until the green color had disappeared. For subsequent enzyme treatment the proteinase K activity was inhibited by incubation in TE-buffer (10 m*M* TRIS.HCl, 10 m*M* EDTA, pH = 7.5) supplemented with 1 m*M* PMSF (V = 15 ml per 8 plugs) for 12 h at 50 °C. Restriction enzyme digestion of high molecular weight DNA was performed according to Van Daelen et al (1989) using 30 units of restriction enzyme per plug. The enzyme was added in three portions over a total incubation time of 6 h.

Yeast DNA isolation

Total yeast DNA was isolated according to Hoffman and Winston (1987). HMW yeast DNA was isolated essentially according to Schwartz and Cantor (1984) with one modification: after incubation in proteinase K/EDTA/sarkosyl the agarose plugs were incubated twice in 10 ml/plug 10 m*M* TRIS.HCl, 10 m*M* EDTA, pH 8.0 for 30 min at 50 °C.

Pulsed field gel electrophoresis

Pulsed field separations of HMW plant DNA were performed using a Rotaphor devise (type IV, Biometra). Restriction fragments in the size range of 100–1,000 kilobasepairs (kbp) were separated using a linear increasing pulse time of 50–70 s at 180 V during a run of 20 h. The angle between the two fields was 120°. The agarose gels (1%, SeaKem UltraPure, FMC) were run in 0.25 × TBE buffer at 14 °C.

Size selection of HMW plant DNA for cloning purposes was carried out by CHEF electrophoresis using a BioRad CHEF DRII apparatus. Gels (1% agarose) were run in 0.5 × TBE (1 × TBE = 90 m*M* TRIS, 90 m*M* boric acid, 2 m*M* EDTA, pH 8.3) at 200 V, 14 °C. Prerunning of the embedded DNA was carried out for 5 h using a pulse time of 60 s. Size selection after partial *Eco*RI digestion was performed by a 2-h-long gel run with a pulse time of 6 s.

The separation of yeast chromosomes was carried out under the same conditions using a pulse time of 60 s during a gel run of 18 h.

Southern analysis

For Southern analysis cloned DNA fragments were purified by agarose gel electrophoresis (SeaKem GTG, FMC), isolated, and labelled by random priming (Random primed DNA labelling kit, USB) with [^{32}P]αdATP. Prior to Southern blotting the DNA in the agarose gels was nicked by UV-treatment (5 s, UV-crosslinker, Hoefer). The washing stringencies used after hybridization were 1×SSC, 1% SDS at 65 °C.

Dot blots on Hybond N$^+$ (Amersham) were prepared using a Schleicher & Schuell Minifold I dot blotter. Transfer and binding of DNA was carried out according to the manufacturer's protocol.

Partial *Eco*RI digestion of HMW plant DNA

Before digestion with *Eco*RI, the low molecular weight (LMW) DNA fraction was removed from the embedded plant DNA by pre-running the agarose plugs on a CHEF gel. Next, the plugs were recovered from the gel and incubated for 2×1 h on ice in 1 ml/plug of restriction buffer (*Eco*RI buffer supplemented with 100 µg/ml BSA, 8 m*M* spermidine.HCl, 1 m*M* DTT). The plugs were transferred to restriction buffer (1 ml/plug) containing 0.5 U *Eco*RI/ml and incubated for a further 30 min on ice. Subsequently, digestion of the DNA was carried out by incubating the plugs for 1 h at 37 °C, and the reaction was terminated by transfer of the plugs into 10 ml/plug of ice-cold 0.5 *M* EDTA, pH 8.0.

To remove restriction fragments smaller than approximately 100 kb, the agarose plugs were loaded on a CHEF gel and electrophoresis was carried out using pulse times of 6 s over 2 h. After electrophoresis, the plugs were recovered from the gel and either stored in 0.5 *M* EDTA pH 8.0 at 4 °C or directly used for cloning.

Construction of the library

Agarose plugs (volume = 100 µl) containing partially *Eco*RI-digested HMW plant DNA were incubated for 2×1 h in 25 ml/plug 10 m*M* TRIS.HCL pH 8.0, 1 m*M* EDTA, 50 m*M* NaCl at room temperature. Next, the DNA was liberated from the plugs by melting the agarose at 68 °C for 10 min and incubated at 40 °C for 10 min. To this molten agarose was added 6 µl β-agarase, and the mixture was stirred very gently with a pipet tip. Digestion of the agarose was carried out during a 5-h incubation at 40 °C. The DNA solution was then allowed to cool down for 5 min on ice, whereafter 10 µl 10×ligation buffer (500 m*M* TRIS.HCl pH 7.5, 100 m*M* $MgCl_2$, 200 m*M* DTT, 10 m*M* ATP, 500 µg/ml BSA), 5 µl T4 DNA ligase (2000 U), and 5 µl (5 µg) dephosphorylated pYAC4-arms were added. The mixture was stirred very gently with a pipet tip, and the DNA was ligated during an overnight incubation at 14 °C. After ligation, the DNA was split into 8 portions, and each portion was used to transform 100 µl (5×10^7 cells) of AB1380 spheroplasts as described (Burgers and Percival 1987; Martin et al 1992). After transformation each batch of yeast cells was split in 4 portions and each portion was plated directly on double-selective (Ura$^-$,Trp$^-$) medium. In a typical experiment, 2,000–3,000 transformants were obtained per DNA plug after 3 days of incubation at 30 °C. For storage, single colonies were re-streaked on double-selective plates and grown for 3 days. Subsequently, cell material was transferred to 96-well microtiter plates, and the yeast clones were cultured for another 3 days on single-selective (Ura$^-$) medium at 30 °C. The cultures were then supplemented with an equal volume of 2×YPD/40% glycerol and stored at −80 °C.

Yeast colony filter preparation

A 96 prong device was used to replicate the YAC library on Hybond N$^+$ (Amersham) filters placed on YPD medium. After an overnight incubation at 30 °C, the filters were transferred to petri dishes containing three layers of Whatmann 3MM paper soaked with 1 *M* sorbitol, 0.1 *M* sodium citrate pH 5.8, 10 m*M* EDTA pH 7.5, 30 m*M* β-mercaptoethanol, and 20 U/ml lyticase. The dishes were sealed with parafilm and incubated overnight at 30°C. Next, the filters were incubated on 3MM paper soaked with 10% SDS (10 min) followed by an incubation on 0.5 *N* NaOH (2×10 min). Neutralization of the filters was carried out by submersion in large excesses of 1.5 *M* NaCl, 1 *M* TRIS.HCl pH 7.5 (10 min.) and 0.15 *M* NaCl, 0.1 *M* TRIS.HCl pH 7.5 (10 min.). Any remaining cell debris was removed from the filters using Kleenex tissues, whereafter the filters were baked for 2 h at 80 °C.

Results

Construction and organization of the library

DNA prepared from agarose-embedded AN5-203b protoplasts appeared to consist of a large fraction (ca. 50%) of LMW DNA of a size smaller than 350 kb. Since this fraction hampered the partial *Eco*RI digestion, the agarose plugs were pre-run for 5 h on a CHEF gel using a pulse time of 60 s. The plugs recovered from the CHEF gel were seen to contain only HMW DNA of a size larger than 3 Mbp. The DNA in these plugs was subjected to a partial *Eco*RI digestion, whereafter the fraction comprising *Eco*RI restriction fragments smaller than approximately 100 kb was removed from the plugs by CHEF electrophoresis using a pulse time of 6 s over 2 h. The remaining HMW *Eco*RI fragments were purified from the agarose plugs and ligated to a 50-fold molar excess of *Eco*RI-digested pYAC4 arms (Burke et al 1987). The ligated DNA was used to transform *S. cerevisiae* AB1380, and transformants were directly selected on URA$^-$ TRP$^-$ plates. In a typical experiment, 1 plug of embedded AN5-203b DNA (6 µg) yielded between 2,000 and 3,000 transformants. A total of 20,112 YAC clones was obtained, and these were grown individually on single selective medium (URA$^-$) in 210 96-well microtiter dishes and stored in duplo at −80 °C.

For screening purposes, filter replicates on Hybond N$^+$ were prepared for each of the 210 microtiter plates. In addition, total yeast DNA was isolated from the pooled yeast clones from every microtiter plate, resulting in 210 DNA pools each containing the DNA from 96 YAC clones. From these pools dot-blots were prepared and, also, 20 superpools were assembled, each containing the DNA from 1,820 clones. The organization of the library in filter replicates of the microtiter plates and dot-blots of the pooled DNAs enables a quick screening of the library by hybridization, whilst the organization in DNA pools and superpools ensures a rapid screening by PCR (Green and Olson 1990).

Characterization of the library

To assess the average size of the YACs in the library, 54 randomly picked yeast clones were analyzed by CHEF electrophoresis. In all cases the presence of artificial chromosomes could be demonstrated either by ethidium bromide staining of the CHEF gels (Fig 1 a) or by Southern hybridization using pBR322 as a probe (Fig 1 b). In approximately 4% of the clones 2 YACs were observed, which can be attributed either to co-transformation events

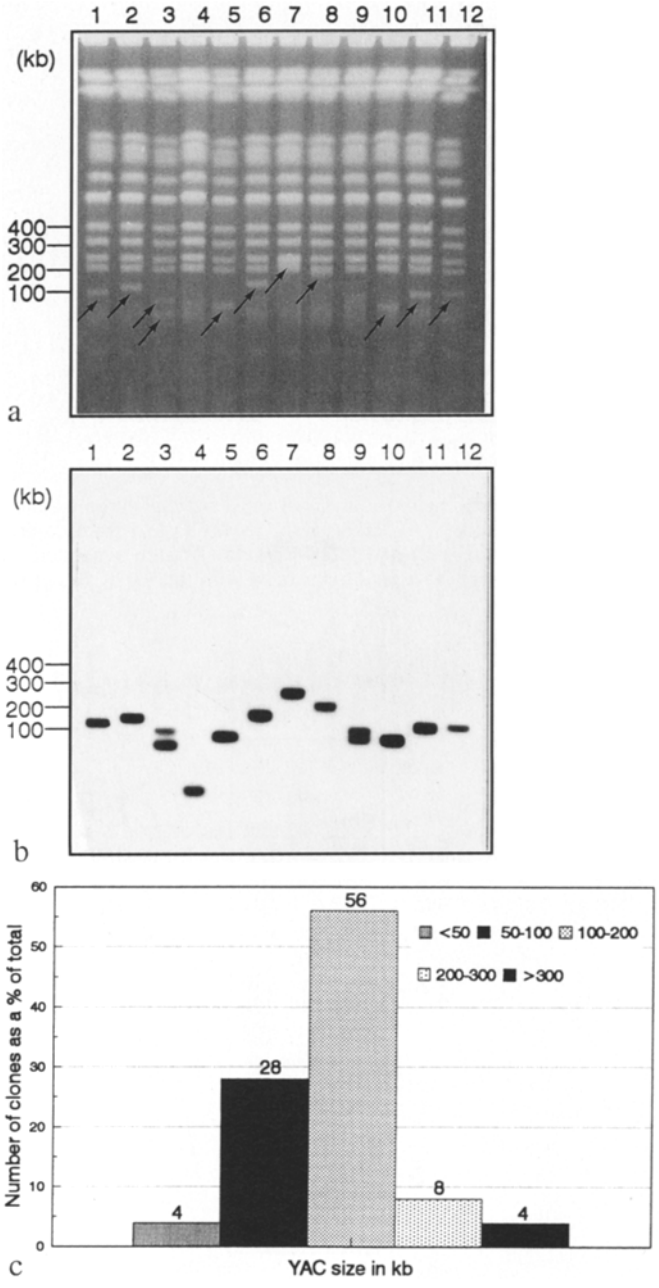

Fig. 1 a Chromosomes of 12 YAC clones from the AN5-203b library resolved by CHEF electrophoresis. The artificial chromosomes are indicated by *arrows*. **b** Hybridization of the yeast artificial chromosomes shown in **a** with pBR322. **c** Size distribution of artificial chromosomes from 54 yeast clones randomly picked from the library

or by the fact that 2 yeast colonies were picked up simultaneously. The average size of the YACs was determined to be 140 kb, with a maximum size of 550 kb. Most of the YACs fell into the size class of 100–200 kb (Fig 1 c). Although a size selection had been applied for clones greater than 100 kb, 32% of the clones contained YACs smaller than 100 kb.

Based on a sugar beet haploid genome size of 750 Mbp (Arumuganathan and Earl 1991) this library represents 3.3 haploid *B. vulgaris* genome equivalents. However, since AN5-203b is a monosomic fragment addition, statistically only 1.6 equivalents of the *B. patellaris* chromosome-1 fragment are present in the library.

The amount of organelle DNA containing YAC clones was determined by hybridizing filter replicates of 16 microtiter plates with the chloroplast specific probe pPHcPs1 (rubisco large subunit) (Van Grinsven et al 1986) and with the mitochondrion specific COX-II probe (cytochrome *c* oxidase II) (Fox and Leaver 1981). Of the clones analyzed 12% appeared to hybridize with the chloroplast specific probe, whereas 0.2 percent of the clones reacted with the COX-II probe. The library was further analyzed by hybridization with the *B. vulgaris* specific satellite DNA probe p663 (C.Jung, unpublished). This satellite sequence was found to be present in 0.4% of the clones (results not shown).

Screening with Sat-121

The satellite DNA Sat-121 was isolated by genomic subtraction from the nematode resistant fragment addition AN5-90 (Salentijn et al 1992). This satellite, with a core sequence of 159 bp, is a member of a family of satellite DNAs that are present exclusively in wild beets of the section *Procumbentes* (Jung et al 1992; Schmidt et al 1990). The distribution of this satellite on the *B. patellaris* fragment in AN5-203b was studied by pulsed field gel electrophoresis of HMW AN5-203b DNA after its complete digestion with *Eco*RI. For comparison, *Eco*RI digested HMW DNA isolated from the fragment additions AN5-90 and AN5-109 (Salentijn et al 1992) was also analyzed. These fragment additions harbor *B. patellaris* chromosomal fragments of 8 Mbp and 13 Mbp, respectively, that carry the nematode resistance gene. Southern analysis using the Sat-121 core sequence as a probe showed that the bulk of this core sequence is present on a single 250-kb *Eco*RI fragment in AN5-203b (Fig. 2, lane a). A strongly hybridizing 250-kb *Eco*RI fragment also appeared to be present in the two other fragment additions (Fig.2, lanes b and c).

The YAC library was screened for the presence of the Sat-121 satellite by hybridization of the dot-blots prepared from the 210 DNA pools using the satellite core sequence as a probe. One pool, derived from microtiter plate no. 5, reacted very strongly with the probe (Fig. 3). The filter replicate prepared from plate no.5 was subsequently screened with Sat-121, which identified clone 5B11 as the hybridizing YAC clone (not shown). This clone was analyzed by CHEF electrophoresis using 2 clones (56G11 and 202D3) as a control. The latter clones were isolated from the library after screening with the *B. vulgaris* specific satellite probe p663 and were known to contain artificial chromosomes of 80 kb that react strongly with the *B. vulgaris* satellite (not shown). Ethidium bromide staining of the CHEF gel showed clearly the presence of the YACs in the control lanes (Fig. 4a, lanes 2 and 3). In the lane containing the 5B11 chromosomes (lane 1) an approximate 250-kb YAC appeared to be present in between the 2 smallest *S.*

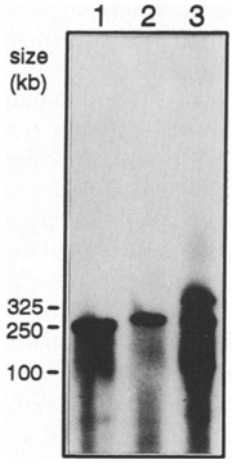

Fig. 2 Pulsed field gel analysis of fragment additions. High-molecular weight DNA isolated from AN5-203b (*lane 1*), AN5-90 (*lane 2*), and AN5-109 (*lane 3*) was digested with *Eco*RI, separated by Pulsed field electrophoresis, and hybridized with the Sat-121 core sequence

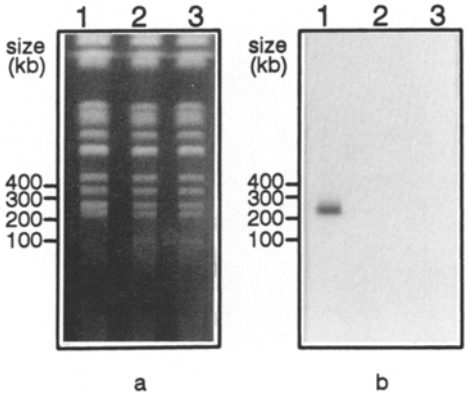

Fig. 4 a, b Separation and analysis of yeast artficial chromosomes. Chromosomes from YAC clone 5B11 (*lanes 1*) and from control clones 56G11 (*lanes 2*) and 202D3 (*lanes 3*) were separated by CHEF electrophoresis (**a**) and hybridized with the Sat-121 core sequence (**b**)

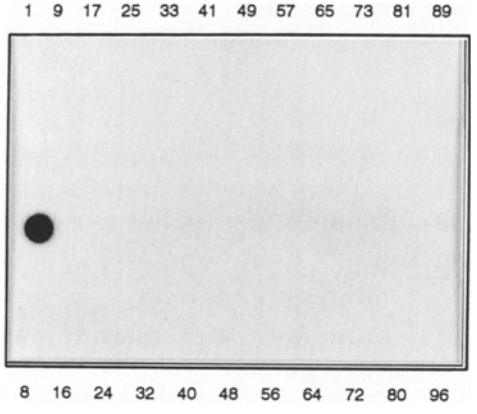

Fig. 3 Screening of part of the YAC library with the Sat-121 core sequence. A dot blot containing 96 DNA pools each consisting of DNA isolated from 96 individual yeast clones was hybridized with the Sat-121 core sequence. (Exposure time 18 h)

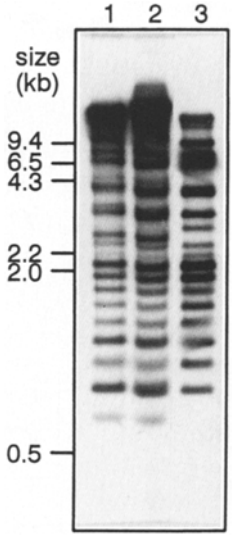

Fig. 5 Analysis of YAC clone 5B11. DNA from clone 5B11 was digested with *Nco*I, separated by electrophoresis, and hybridized with the Sat-121 core sequence (*lane 3*). As a control *Nco*I-digested DNA from the fragment additions AN5-120 (*lane 1*) and AN5-203b (*lane 2*) was used

cerevisiae chromosomes (225 and 295 kb). The presence of this YAC was confirmed by Southern hybridization using the Sat-121 core sequence as a probe. In the lane containing 5B11 DNA a very strong hybridizing fragment was present that had a size of 250 kb (Fig. 4b), whereas no hybridization was evident with the 2 control YACs.

Organization of Sat-121 on the YAC 5B11

To study the organization of the satellite DNA on YAC 5B11, Southern analysis was carried out on total 5B11 DNA after digestion with *Nco*I. For comparison, NcoI digested DNA from AN5-203b and AN5-120 (a nematode resistant fragment addition carrying a similar *B. patellaris* fragment as AN5-203b) was used. After hybridization with the Sat-121 core sequence, both fragment additions showed an identical ladder pattern that is characteristic for in-tandem arranged satellite DNAs (Horz and Zachau 1977; Schmidt and Metzlaff 1991) (Fig. 5, lanes 1 and 2). The ladder pattern starts with an NcoI-fragment of approx. 320 bp (two repeat units) and increased with steps of approximately 160 bp (one repeat unit) up to *Nco*I fragments

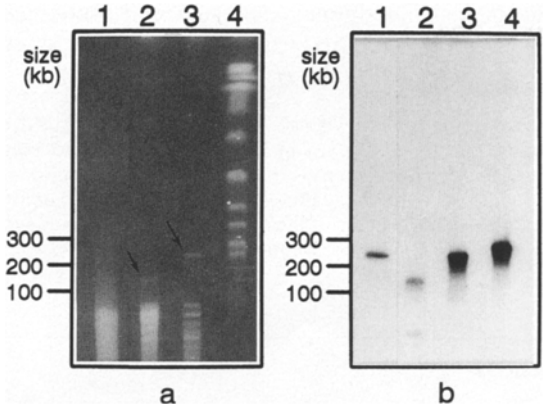

Fig. 6 a, b Pulsed field analysis of YAC clone 5B11. High molecular weight DNA isolated from YAC clone 5B11 was restricted with *Nco*I (*lanes 2*) and *Eco*RI (*lanes 3*), separated by CHEF electrophoresis (**a**), and hybridized with the Sat-121 core sequence (**b**). As a control *Eco*RI-restricted AN5-203b DNA (*lanes 1*) and undigested 5B11 DNA (*lanes 4*) were used

of 2 kbp in size. Hereafter, the size the *Nco*I fragments increased further to an estimated size of 20 kbp, but the spacing between the different fragments became more variable. The ladder pattern obtained for YAC clone 5B11 appeared to be almost identical to the patterns of the fragment additions (Fig. 5, lane 3). Only a few rungs of the fragment addition ladders were either absent or less intense in the YAC-encoded ladder, confirming that the majority of the Sat-121 core sequences present in AN5-203b had been cloned into YAC clone 5B11.

The organization of the repeat DNA in YAC 5B11 was further analyzed by pulsed field electrophoresis (Fig. 6 a). HMW DNA was isolated from the YAC clone 5B11, digested with *Eco*RI (lane 3) and *Nco*I (lane 2) and separated on a CHEF gel. As controls, *Eco*RI digested AN5-203b DNA (lane 1) and undigested HMW DNA isolated from clone 5B11 (lane 4) were used. After staining of the gel an approximate 250-kb *Eco*RI restriction fragment was clearly visible in the lane containing the *Eco*RI digested 5B11 DNA (see arrow, lane 3). In the lane containing the *Nco*I digested 5B11 DNA a 150-kb fragment had been separated from the bulk of the restricted DNA (see arrow lane 2). The DNA in the CHEF gel was transferred to a filter and hybridized with the satellite core sequence (Fig. 6 b). In lane 3 a strongly hybridizing 250 kb *Eco*RI fragment was detected, showing that the fragment visible in Fig.6 a, lane 3, was the *Eco*RI insert present in YAC 5B11. A hybridizing *Eco*RI fragment of exactly the same size as the YAC insert was present in the lane containing the digested AN5-203b DNA (lane 1), showing that the satellite cluster had been cloned without deletions or rearrangements into the YAC vector.

The difference in mobility between the intact YAC (Fig. 6 b, lane 4) and the insert must be attributed to the presence of the YAC arms (10 kb). However, it can not be excluded that, in addition to the 250-kb *Eco*RI fragment, some *Eco*RI fragments of low molecular weight were present in the YAC, which might contribute to the size difference between the intact YAC and the 250 kb fragment. The 150 kb *Nco*I fragment visible in lane 2 also appeared to hybridize with Sat-121 and thus was derived from the YAC insert. The weaker hybridization of this fragment compared to that of the 250-kb *Eco*RI-fragment showed that the 150-kb *Nco*I fragment contained a relatively small number of repeat units.

To analyze YAC 5B11 for the presence of CpG islands, and thus for a possible presence of open reading frames (Bird 1986), HMW 5B11 DNA was digested with *Mlu*I, *Cla*I, *Sal*I, *Xho*I and *Hpa*II and analyzed by Southern hybridization using Sat-121 as a probe (results not shown). However, the YAC appeared to contain (unique) restriction sites for *Cla*I and *Hpa*II only and was not restricted by the other enzymes used. From these results it could not be inferred conclusively whether or not CpG islands were present on the YAC.

Discussion

In this paper the construction of a AN5-203b YAC library is described that will be used in the isolation of the beet cyst nematode-resistance gene encoded by the $Hs1^{pat-1}$ locus in *B. patellaris*. The library consists of approximately 20,000 clones with an average YAC size of approximately 140 kb. This average size is relatively small when compared to YAC libraries constructed of human or rodent DNA (Anand et al 1990; Chumakov et al 1992; Deloukas et al 1992; Larin et al 1991), which have average insert sizes ranging from 350 kb up to 1 Mbp, but is similar to the sizes reported for most of the plant YAC libraries constructed (Edwards et al 1992; Eyers et al 1992; Grill and Somerville 1991; Kleine et al 1993; Martin et al 1992; Ward and Jen 1990). The only exception probably forms the *Arabidopsis* Yup library (Ecker 1990) with an average YAC size of 250 kb.

Attempts to increase the average YAC size in the AN5-203b library were unsuccessful, since selections on pulsed field gels of DNA fragments larger than approximately 500 kb always resulted in an estimated 100-fold drop in the overall cloning efficiency. This phenomenon has also been observed for barley DNA (Kleine et al 1993) and probably reflects the general difficulty to obtain sufficient amounts of clonable HMW plant DNA.

The percentages of organelle DNA containing clones in the constructed library (12% chloroplast (cp) DNA and 0.2% mitochondrial (mt) DNA) also are in the same order of magnitude as those reported for most plant YAC libraries. Although the agarose plugs were pre-run on a CHEF gel to remove DNA molecules smaller than approximately 3 Mbp, a substantial amount of the 147 kb sugar beet cpDNA (Brears et al 1986) was apparently retained within the plugs. This can be explained by the fact that in the agarose a large fraction of the cpDNA molecules will be

present as circles, which migrate hardly at all during pulsed field gel electrophoresis. In contrast, most of the mtDNA molecules will be present in a linear form in the agarose plugs (Bendich and Smith 1990), resulting in a small percentage of mtDNA containing clones in the library.

Screening of the AN5-203b library with the *B. vulgaris* specific satellite p663 showed that 0.4% of all clones react strongly with this repeat sequence. This is in good agreement with results obtained for the sugar beet YAC library (Eyers et al 1992), where 0.4% of the clones were found to react with either the *Bam*HI (Schmidt and Metzlaff 1991) or the *Eco*RI (Schmidt et al 1991) monomeric unit sequences of two different sugar beet satellite DNAs.

On the 12 Mpb *B. patellaris* fragment present in AN5-203b a cluster of satellite DNAs is present on a 250-kb *Eco*RI restriction fragment. This satellite, referred to as Sat-121, is highly homologous to satellites isolated from *B. procumbens* (Jung et al 1992; Schmidt et al 1990) and belongs to a family of satellite DNAs specific to wild beets of the section *Procumbentes* (Salentijn et al. submitted). As the Sat-121 containing 250-kb *Eco*RI fragment has been shown to be present in eight different nematode resistant fragment additions of *B. patellaris* chromosome 1 in *B. vulgaris* (Salentijn, unpublished), this cluster is probably tightly linked to the nematode resistance gene. Screening of the library with Sat-121 yielded 1 strongly hybridizing YAC clone (5B11). This frequency (1 in 20,000) was in the order of expectation since the library represents 1.6 addition fragment equivalents. Analysis of this clone showed that it contains a 250 kb artificial chromosome. The typical ladder pattern obtained after *Nco*I digestion of 5B11 DNA and hybridization with the Sat-121 core sequence was almost identical to the ladder pattern obtained with AN5-203b DNA, indicating that most of the Sat-121 repeat units present in AN5-203b are cloned in YAC 5B11. Pulsed field analysis of this YAC showed that in addition to the ladder of LMW *Nco*I fragments, there is a single 150 kb *Nco*I fragment present. According to the low level of hybridization of this fragment with the repeat core sequence, this fragment must harbor a relatively small amount of Sat-121 units. Since no *Eco*RI restriction sites are present within this 150-kb fragment, the Sat-121 units on the 150-kb *Nco*I fragment are probably embedded in other types of repeated DNA. The remainder of the YAC clone (100 kb) most likely constitutes of 600–700 Sat-121 units arranged in tandem, as evidenced by the strongly hybridizing *Nco*I ladder.

It is known that the presence of repetitive DNA on the YACs can cause instability of the artificial chromosomes (Neil et al 1990). In barley, for instance, it was found that the presence of the repetitive WIS-2 and BIS-1 elements cause rearrangements in yeast artificial chromosomes (Dunford et al 1993). However, there have been no indications so far that YAC 5B11 is not stable, demonstrating that this highly repetitive plant DNA of 250 kb can be maintained stably in *S. cerevisiae* AB1380.

The tight linkage of the Sat-121 satellite cluster on the 250-kb *Eco*RI fragment to the nematode resistance gene makes it a suitable starting point for a chromosomal walk towards the resistance gene. At present, end-probes are being isolated from YAC 5B11 that will be used to assemble a YAC contig spanning the $Hs1^{pat-1}$ locus.

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