Use of Species-Specific Satellite DNA from *Bursaphelenchus xylophilus* as a Diagnostic Probe

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ABSTRACT

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We describe the development of a species-specific DNA probe with the satellite DNA (satDNA) isolated from the Japanese J10 isolate of *Bursaphelenchus xylophilus*. We show that this *Msp*I satDNA probe is effective directly on a single squashed nematode spotted onto a filter. Therefore, in the pinewood nematode species complex, the identification of the plant pathogenic species *B. xylophilus* may be accomplished both rapidly and reliably. We also show that the *Msp*I satellite sequence is polymorphic within *B. xylophilus* species. Hybridization patterns of *B. xylophilus* isolates with the *Msp*I satellite probe result in very different profiles, with polymorphisms characterizing each of the tested strains. The most important polymorphism, involving mainly qualitative differences, was observed in the hybridization ladder from 1.6 kb upward. Furthermore, the American and Japanese isolates hybridized more strongly to the probe than did the Canadian isolates. Therefore, Japanese isolates of *B. xylophilus* seem to be closer to the American than to the Canadian isolates. This result agrees with those previously obtained with other DNA probes. This observation supports the hypothesis that a *B. xylophilus* isolate reached Japan from North America, probably from the United States.

Additional keywords: fingerprinting.

The nematode *Bursaphelenchus xylophilus* is the causal agent of pine wilt disease (18,22). This nematode has been identified in Japan (18) and is reported to occur in the United States (36), Canada (19), and China (20,37). It is difficult to identify some isolates of *B. xylophilus* and a closely related species *B. mucronatus*, which is not pathogenic to pine trees under field conditions (17,21). *B. mucronatus* differs from *B. xylophilus* only by the presence of a mucron on the female tail. However, a North American *B. xylophilus* isolate, US10, is morphologically similar to *B. mucronatus* (36). A third species, *B. fraudulentus* (27), is morphologically similar to *B. mucronatus* but does not mate with *B. mucronatus* and *B. xylophilus* (29). These three species are thought to be derived from common origins, and, therefore, it has been proposed that *B. xylophilus, B mucronatus,* and *B. fraudulentus* constitute a supraspecies referred as "the pinewood nematode species complex" (PWNSC) (12,35).

Attempts have been made to distinguish between nematode species and pathotypes, using morphological characteristics (24), protein profiles (9,13), monoclonal antibodies (30), and enzyme electrophoresis (9). The protein content of the nematode is subject to environmental and developmental variation, and Bakker (3) showed that protein variation between pathotypes is often less than that observed within pathotypes.

Recently, DNA probes have been used with some success to identify nematodes, such as *Meloidogyne* (7,8,26). DNA hybridization (1,33), restriction fragment length polymorphism (RFLP) analysis (6), sequence differences in ribosomal genes (35), and polymerase chain reaction (4,15) have been used in studies of the PWNSC.

In this paper, we describe the development of a species-specific DNA probe. This sequence, isolated from *B. xylophilus*, belongs to a satellite DNA (satDNA) that constitutes up to 30% of the nematode genome (34). It is represented as a tandemly repeated *Msp*I site-containing sequence with a monomeric unit of 160 bp. Thirteen monomers were cloned and sequenced. The consensus sequence is 62% A+T rich, with the presence of direct, indirect, and invert repeat clusters (34). Analysis of monomer sequences shows on-average divergence of 3.9% from the calculated consensus. This variability could provide a valuable tool for disclosing evolutionary relationships among species. In this study, we identified different isolates of *B. xylophilus* and demonstrated that each has a characteristic profile. Furthermore, because this sequence is highly reiterated in the genome, it can be used as a sensitive and reliable probe for direct identification of a *B. xylophilus* individual, using a simple squashed nematode procedure.

MATERIAL AND METHODS

Nematode isolates. Eight strains of *B. xylophilus* were used for this study. The name and geographic origin of each strain are reported in Table 1. All of these strains were cultured monoxenically on *Botrytis cinerea* grown on crushed corn kernels (33). Culture tubes of *Botrytis cinerea* were inoculated with strains of *Bursaphelenchus* and stored in an incubator at 25 C for 1 mo. After this time, all the mycelium disappeared, and most of the nematodes had stopped their development at the fourth juvenile stage (J4), which appeared to be the "resistant stage" in culture. Nematodes were extracted by pouring the contents of the tubes onto tissue paper supported by a 1-mm sieve placed in a dish with water. After 1 day, the nematodes were collected in the water, pelleted in a microcentrifuge, and stored at −80 C until use.

DNA isolation. Nematodes from each population were frozen in liquid nitrogen and ground with a mortar and pestle. The DNA was extracted from the resulting powder by a phenol/chloroform procedure (28). After ethanol precipitation, DNA was resuspended in 10 mM Tris (pH 8.0) and 1 mM EDTA and stored at −20 C.

Electrophoresis, Southern blot, labeling, and hybridization procedures. Genomic and plasmid DNA were digested to completion with restriction endonucleases from Boehringer Mannheim (Meylan, France) as recommended by the manufacturer. Electrophoresis of digested DNA samples and processing for transfer to nylon membranes were done following standard procedures (32). The satellite probe, consisting of the *Msp*I satDNA sequence (34),

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was radioactively labeled with ^{32}P by the random oligonucleotide primer method (14). All hybridizations were conducted overnight at 65 C. The hybridization buffer consisted of 6× SSC (1× SSC = 0.15 M sodium chloride, 0.0015 M sodium citrate, pH 7.0), 5× Denhardt's solution (Ficoll 400 at 0.5 g/L, polyvinylpyrrolidine at 0.5 g/L, bovine serum albumin at 0.5 g/L), 0.5% sodium dodecyl sulfate (SDS), and calf-thymus DNA at 25 mg/ml. After hybridization, filters were washed at 65 C in 2× SSC and 0.1% SDS and then in 1× SSC and 0.1% SDS. After posthybridization washes, filters were exposed to X-ray film with an intensifying screen at −80 C. This procedure was repeated at least two times.

Detection of satellite sequences by direct hybridization of a single nematode. The presence of *Msp*I satDNA sequences in single squashed nematodes was investigated by hybridization (2). One individual nematode was placed on a nylon filter sheet. The nematode was ruptured by gentle pressure exerted with a yellow, flat-tipped micropipet tip, which is sufficiently translucent to view the nematode and verify lysis. Nematodes squashed on a nylon filter were lysed by layering, successively, the filter on Whatman 3MM papers (Whatman International, Ltd., Maidstone, England) soaked with 10% SDS (2 min), 0.5 M NaOH/2.5 M NaCl (two times, 5 min each), and 3 M sodium acetate (pH 5; three times, 2 min each). The filter was dried at room temperature (30 min) and baked (80 C, 1 h). Prehybridization and hybridization were carried out as described above. The procedure was repeated three times to verify reproducibility.

RESULTS

Specificity of the *Msp*I satellite sequence. The *Msp*I satellite sequence was previously isolated by digesting total DNA from the Japanese J10 strain of *B. xylophilus* with *Msp*I restriction enzyme. Numerous copies of the 160-bp *Msp*I fragment isolated from the gel were cloned and sequenced (34). The consensus sequence of this satellite family is shown in Figure 1.

Figure 2 shows an autoradiogram of genomic DNA from the *B. xylophilus, B. mucronatus,* and *B. fraudulentus* isolates digested with the *Msp*I and hybridized with the cloned 160-bp *Msp*I fragment. This allowed us to clearly separate the *B. xylophilus* species from the two other species. The genomic DNA of all *B. xylophilus* isolates showed the presence of a ladder of multimers of the 160-bp *Msp*I repeats, which is typical of the satellite sequences arranged in tandem arrays. This probe, isolated from the Japanese *B. xylophilus* J10 isolate (34), displayed weaker hybridization with DNA of the two Canadian isolates of *B. xylophilus* than did the other Japanese and American isolates.

Use of the *Msp*I satellite sequence for fingerprinting *B. xylophilus* isolates. Polymorphisms in the hybridization ladder pattern were generated by the *Msp*I satDNA probe on genomic DNA of six *B. xylophilus* isolates digested to completion with *Alu*I, *Dra*I, *Hae*III, and *Hin*dIII (Fig. 3). Because no qualitative differences were detected between their hybridization ladder patterns until the decamer unit (1.6 kb; data not shown) and the most important polymorphism, involving mainly qualitative hybridization differences observed in the ladder from the undecamer upward, Figure 3 shows only the hybridization ladder pattern between 1.6 and 9 kb.

In the *Dra*I digestion, the absence of signal for the oligomer n=11 (indicated by A) in the ladder hybridization pattern differentiated the Canadian isolate Bc from the other tested isolates. Nevertheless, extensive differences were observed in the different hybridization ladder patterns of the tested *B. xylophilus* isolates (Fig. 3A).

In the *Alu*I digestion, a lack of hybridization to the oligomer n=13 (indicated by B) was observed for the US9 isolate (Fig. 3B). Strong hybridization with oligomers n=17 and n=21 (indicated by C and D, respectively) differentiated the J10 and K48-4 isolates. The uniqueness of hybridization with the band around 8 kb (indicated by E) and the strong hybridization of a 7.5-kb band (indicated by F) identified the US15 and the US10 isolates, respectively.

In the *Hae*III digestion (Fig. 3C), the absence of hybridization for the oligomer n=12 (indicated by G) and for the oligomer n=16 (indicated by H) was isolate-specific for US10 and K48-4, respectively. The US15 and J10 isolates were separated from the others by the absence of hybridization for the oligomer n=17 (indicated by I), whereas the J10 isolate differed from US15 by the absence of hybridization for the oligomer n=19 (indicated by J). The absence of hybridization from 4.3 to 5 kb in the ladder characterized the US9 isolates.

In the *Hin*dIII digestion (Fig. 3D), the absence of a signal corresponding to the oligomer n=13 (indicated by K) separated the US9 and the Bc isolates from the others. Isolates US9 and Bc could be differentiated by the uniqueness of the hybridization signals with the oligomers n=19 (indicated by L) and n=21 (indicated by M) in the US9 isolate. US15 isolate was characterized by the absence of a signal for the oligomer n=17, (indicated by N). The strong hybridization of the oligomer n=13 (indicated by O) distinguished the J10 and US10 isolates from the other isolates tested, whereas the uniqueness of the presence of the oligomer n=21 in the isolate J10 separated it from the US10 isolate (indicated by P).

Use of a *Msp*I satellite sequence as a rapid diagnostic probe. To determine whether the *Msp*I satellite could be used as a rapid diagnostic probe, we devised a simple direct hybridization method on nematode prints. As shown in Figure 4, the *Msp*I satellite probe hybridized with the individuals of the *B. xylophilus* isolates J10 and Bc. This probe also recognized individuals from all the other isolates of *B. xylophilus* (data not shown). Different numbers of nematodes (one to 20) were tested, and all produced a positive

TABLE 1. Geographic origin and host of the *Bursaphelenchus* isolates used in this study

Isolate	Origin	Host
B. xylophilus		
Bc	Clinton, BC, Canada	*Pinus contorta*
Fids	Houston, BC, Canada	*P. contorta*
US9	Tucson, AZ, U.S.	*P. helepensis*
US10	Cloquet For. Ctr., MN, U.S.	*Abies balsamea*
US15	Cook County, IL, U.S.	*P. sylvestris*
J3	Kumamoto Pref., Ueki, Japan	*P. densiflora*
J10	Fukushima Pref., Nishiaizu, Japan	*P. densiflora*
K48-4	Nakaninami, Japan	*P. thunbergii*
B. mucronatus		
J13	Chiba Pref., Yachiyo, Japan	*P. thunbergii*
BmN	Hanestad, Norway	*P. sylvestris*
B. fraudulentus		
BfHu	Sopron, Hungary	*Quercus petraea*
H37	Erlangen, Germany	*Cerasus avium*

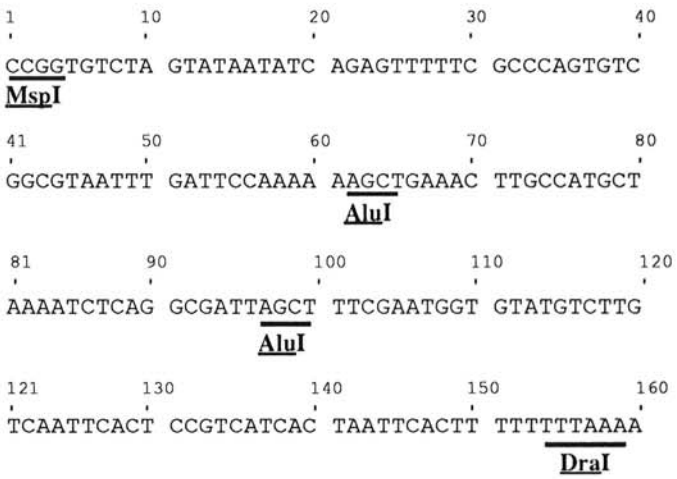


Fig. 1. Nucleotide sequence of the *Msp*I satellite monomer (32). The positions of the restriction-enzyme cut sites are shown as lines below the sequence with the name of the enzymes.

signal. No cross-reaction with *B. mucronatus* (Fig. 4) and *B. fraudulentus* (data not shown) was observed with the *Msp*I satellite probe. Therefore, the species-specific probe detected the presence of only one *B. xylophilus* squashed nematode after overnight exposure of the blot (Fig. 4).

DISCUSSION

In this report, we present evidence that the *Msp*I satDNA sequence isolated from the nematode *B. xylophilus* (34) provides a molecular tool for identification of species belonging to the PWNSC. RFLP can be used to differentiate the species of the PWNSC (1,33,35). However, the normal procedure involves waiting for 2–3 wk for the emergence of adults; this is followed by DNA extraction, restriction enzyme digestion, gel electrophoresis, and Southern blotting, which can take a number of days. Rapid identification of the species and pathotypes of the PWNSC can be accomplished using sequences specific for one pathogen in a simple dot blot procedure.

SatDNAs have been characterized in a number of plant and animal species (5,16,31). They contain identical or closely related sequences repeated thousands of times in tandem arrays. Their abundance can be distributed over 66% of the genome (31). In general, satDNAs are located in heterochromatin and generally are not transcribed (10,23). Due to their ability to exhibit sequence variations, satDNAs offer a good prospect for species and subspecies identification. We previously isolated and characterized a *Msp*I satDNA sequence from *B. xylophilus* (34). It appears to contain up to 30% of the total genomic DNA. Thus, this satellite represents a large part of the *B. xylophilus* genome. This repetitive sequence is not transcribed and exhibits a divergence in the sequenced satellite monomers of 3.9% from the calculated consensus (34).

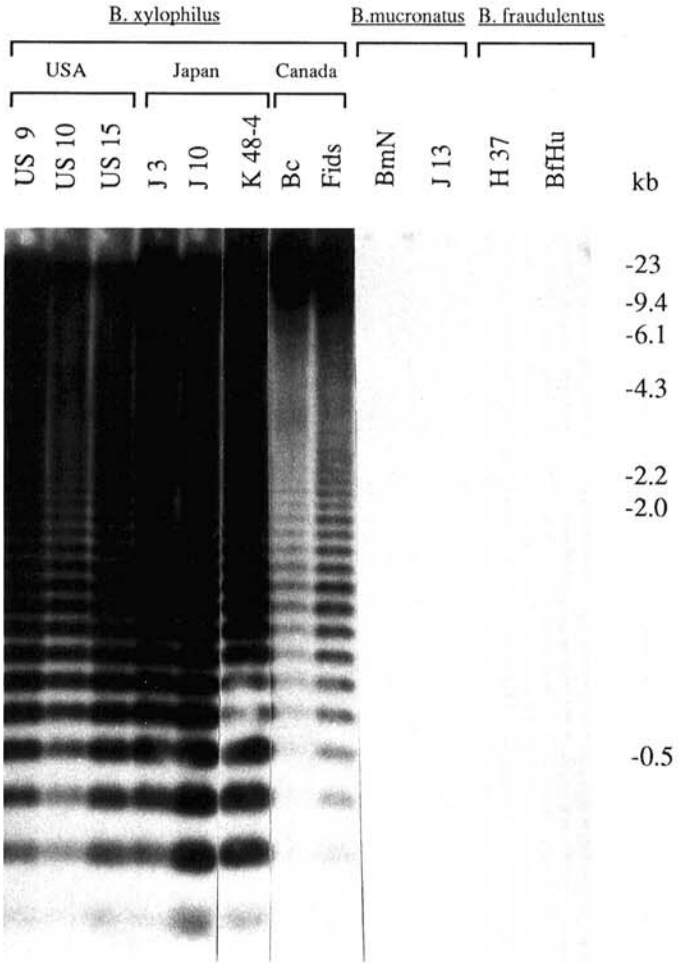


Fig. 2. Autoradiogram of genomic Southern blot of eight *Bursaphelenchus xylophilus* isolates digested by *Msp*I and hybridized with the ^{32}P-labeled *B. xylophilus* J10 satellite-DNA monomer. Each lane of the agarose gel contained equal amounts of DNA. Molecular weight markers are shown in kilobases.

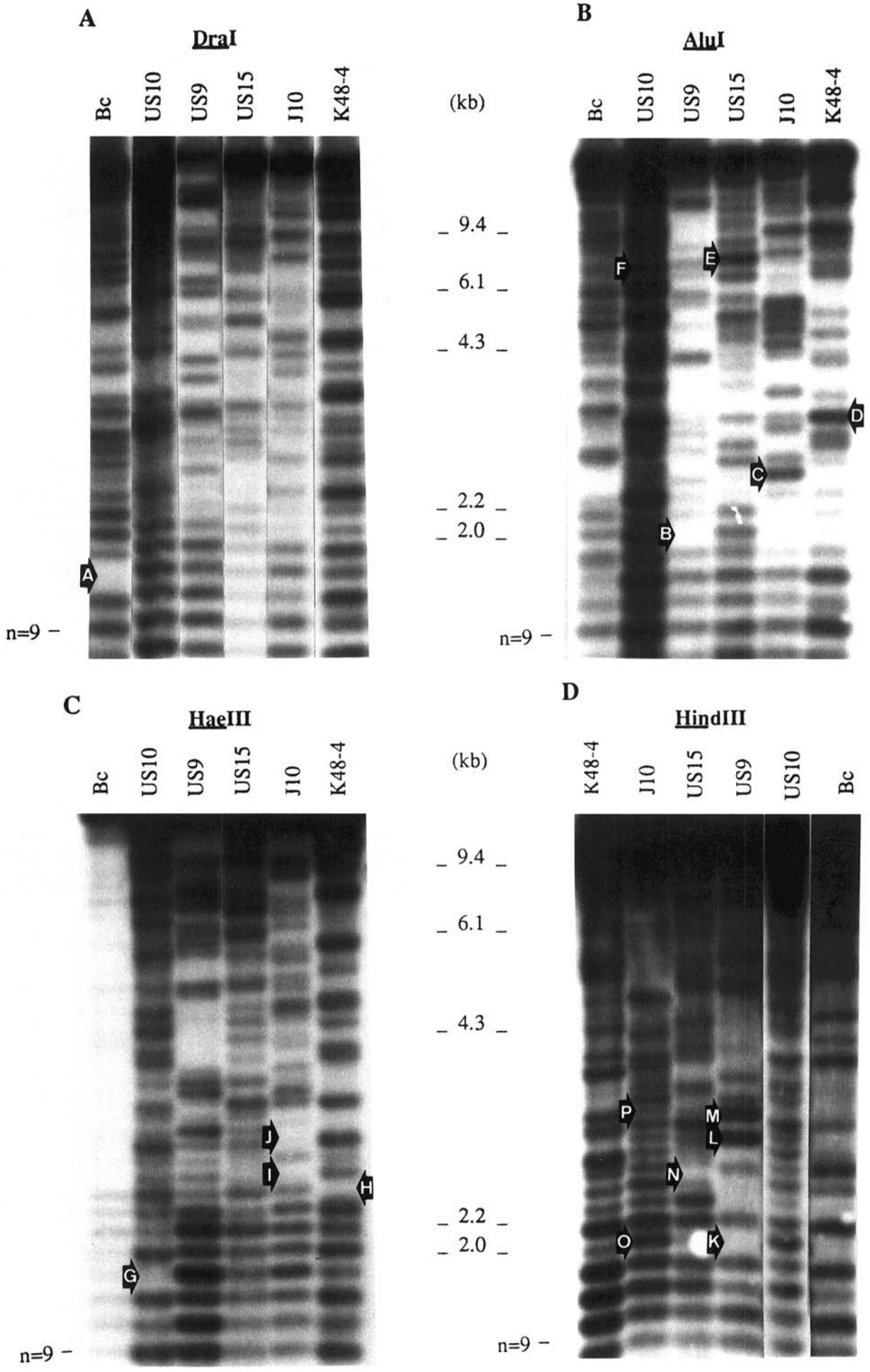
In this *Msp*I satDNA family, if all repeats in a tandem array were identical, DNA digestion to completion with *Msp*I would generate in Southern blot a very simple banding pattern that would include a major band at 160 bp. However, during the evolution of a genome, satDNA turnover occurred by sequence amplification, unequal crossing over, point mutation, and gene conversion. Furthermore, high frequency of point mutations acts on satDNA blocks and induces creation or destruction of some restriction enzyme sites visualized in Southern blot by a typical ladder hybridization. In this ladder pattern, regular periodic spaces of the oligomeric bands were observed, with some bands represented less than others or absent. This residual pattern obtained after complete *Msp*I digestion can be accounted for by the loss of some *Msp*I recognition sites within the tandem repeat. The observed situation for the *Msp*I repeated sequence from *B. xylophilus* was already found in the case of satellite families from the nematodes *Panagrellus redivivus* and *Meloidogyne incognita*, in which similar percentages of sequence divergences were observed (11,25).

The abundance, variability, and species-specificity of the *B. xylophilus* satellite family were used to demonstrate its usefulness in fingerprinting. In Southern blotting experiments, we showed that the American and Japanese isolates hybridized more strongly to the *Msp*I probe than did the Canadian isolates. Therefore, Japanese isolates of *B. xylophilus* seem to be closer to the United States isolates than to the Canadian isolates. This result agrees with those previously obtained with other probes (15,33). This observation supports the hypothesis that a *B. xylophilus* isolate reached Japan from North America (12), probably from the United States.

We observed that the *Msp*I satellite sequence is polymorphic within the *B. xylophilus* species. This was shown in genomic DNA digested with enzymes that did not cut within the repeat unit and probed with the *Msp*I satellite-sequence monomer. The hybridization patterns of digested genomic DNA from *B. xylophilus* isolates did not show qualitative differences in the hybridization ladder until the decamer unit. The most important polymorphisms can be observed between 1.6 and 9 kb. This indicates that these repeat blocks of about 10 units and more are dispersed in different regions of the genome and flanked by sites for these enzymes. Alternatively, sequence variations within the large repeat block may provide sites for these enzymes. Therefore, hybridization patterns of *B. xylophilus* isolates with the *Msp*I satellite probe resulted in very different profiles for each strain. Although, fingerprints were obtained with other molecular markers (1,15, 33,35), our data show that similar results can be obtained with large tandem repeat sequences, such as satDNA. Further studies of tandem repeated sequences within PWNSC might enhance our understanding of the origin and evolution of closely related species and pathotypes.

Furthermore, we showed that the *Msp*I satDNA probe was effective when placed directly on squashed nematodes spotted onto a filter, using a very simple procedure with no need to extract DNA. The use of *Msp*I satDNA as a species-specific probe leaves no doubt as to the identity of the nematode. Therefore, in the PWNSC, the identification of the plant pathogenic species *B.*

Fig. 3. Autoradiogram of a genomic Southern blot of six *Bursaphelenchus xylophilus* isolates digested by four restriction enzymes and hybridized with the ^{32}P-labeled *B. xylophilus* J10 satellite-DNA monomer. **A,** *Dra*I; **B,** *Alu*I; **C,** *Hae*III; and **D,** *Hin*dIII. Molecular weight markers are shown in kilobases. The abbreviation n=9 indicates the position of the monomer in the hybridization ladder. The lettered arrows indicate polymorphisms characterizing each of the tested isolates. The very poor hybridization observed with the Bc genomic DNA digested with *Hae*III was due to a low quantity of DNA.


A
DraI
Bc
US10
US9
US15
J10
K48-4
A
n=9
B
AluI
(kb)
9.4
6.1
4.3
2.2
2.0
F
E
D
C
B
n=9
C
HaeIII
J
I
H
G
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HindIII
P
M
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N
O
K
n=9

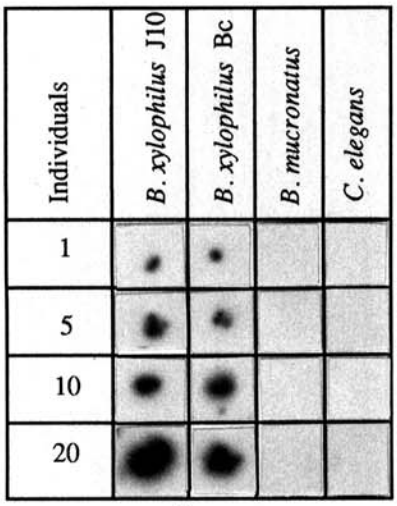

Fig. 4. Direct hybridization of the ^{32}P-labeled *Bursaphelenchus xylophilus* J10 satellite-DNA monomer 2 on different numbers of nematodes belonging to *B. xylophilus* J10 and Bc, *B. mucronatus*, and *Caenorhabditis elegans*.

xylophilus may be carried out both rapidly and reliably. This step may be crucial for the success of a direct detection of *B. xylophilus* in wood samples and will prove to be of great use in ecological and population studies.

LITERATURE CITED

1. Abad, P., Tarès, S., Bruguier, N., and De Guiran, G. 1991. Characterization of the relationships in the pine wood nematode species complex (PWNSC) using heterologous *unc-22* DNA probe from *Caenorhabditis elegans*. Parasitology 102:303-308.
2. Anxolabéhère, D., Nouaud, D., Périquet, G., and Tchen, P. 1985. P-element distribution in Eurasian populations of *Drosophila melanogaster*: A genetic and molecular analysis. Proc. Natl. Acad. Sci. USA. 82:5418-5422.
3. Bakker, J. 1987. Protein variation in cyst nematodes. Ph.D. thesis. Agricultural University, Wageningen, the Netherlands.
4. Beckenbach, K., Smith, M. J., and Webster, J. M. 1992. Taxonomic affinities and intra- and inter-specific variation in *Bursaphelenchus* spp. as determined by polymerase chain reaction. J. Nematol. 24:140-147.
5. Beridze, T. 1986. Satellite DNA. Springer-Verlag, Berlin.
6. Bolla, R. T., Weaver, C., and Winter, E. K. 1988. Genomic differences among pathotypes of *Bursaphelenchus xylophilus*. J. Nematol. 20:309-316.
7. Castagnone-Sereno, P., Piotte, C., Uijthof, J., Abad, P., Wajnberg, E., Vanlerberghe-Masutti, F., Bongiovanni, M., and Dalmasso, A. 1993. Phylogenetic relationships between amphimictic and parthenogenetic nematodes of the genus *Meloidogyne* as inferred from repetitive DNA analysis. Heredity 70:195-204.
8. Curran, J., McClure, M. A., and Webster, J. M. 1986. Genotypic differentiation of *Meloidogyne* populations by detection of restriction fragment length difference in total DNA. J. Nematol. 18:83-86.
9. Dalmasso, A., and Bergé, J. B. 1978. Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: Application to the taxonomy of *Meloidogyne*. J. Nematol. 10:147-154.
10. Davis, C. A., and Wyatt, G. R. 1989. Distribution and sequence homogeneity of an abundant satellite DNA in the beetle, *Tenebrio molitor*. Nucleic Acids Res. 17:5579-5586.
11. De Chastonay, Y., Müller, F., and Tobler, H. 1990. Two highly reiterated nucleotide sequences in the low C-value genome of *Panagrellus redivivus*. Gene 93:199-204.
12. De Guiran, G., and Bruguier, N. 1989. Hybridization and phylogeny of the pine wood nematode (*Bursaphelenchus* spp.). Nematologica 35:321-330.
13. De Guiran, G., Lee, M. J., Dalmasso, A., and Bongiovanni, M. 1985. Preliminary attempt to differentiate pinewood nematodes (*Bursaphelenchus* spp.) by enzyme electrophoresis. Rev. Nématol. 8:85-91.
14. Feinberg, A. P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.
15. Harmey, J., and Harmey, M. A. 1994. Detection of *Bursaphelenchus* species using DNA fingerprinting. J. Nematol. 25:406-415.
16. Jelinek, W. R., and Schmid, C. W. 1982. Repetitive sequences in eukaryotic DNA and their expression. Annu. Rev. Biochem. 51:813-844.
17. Kiyohara, T., and Bolla, R. I. 1990. Pathogenic variability among populations of the pinewood nematode *Bursaphelenchus xylophilus*. For. Sci. 36:1061-1076.
18. Kiyohara, T., and Tokushige, Y. 1971. Inoculation experiments of a nematode, *Bursaphelenchus* spp., onto pine trees. J. Jpn. For. Soc. 53:210-218.
19. Knowles, K., Beaubien, Y., Wingfield, M. J., Baker, F. A., and French, D. W. 1983. The pinewood nematode new in Canada. (Abstr.) For. Chron. 59:40.
20. Li, G. W. 1983. Discovery of and preliminary investigation on pine wood nematodes in China. For. Sci. Technol. 7:15-18.
21. Mamiya, Y., and Enda, N. 1979. *Bursaphelenchus mucronatus* n. sp. (Nematoda:Aphelenchoididae) from pine wood and its biology and pathogenicity to pine tree. Nematologica 25:353-361.
22. Mamiya, Y., and Kiyohara, T. 1972. Description of *Bursaphelenchus lignicolus* n. sp. (Nematoda:Aphelenchoididae) from pine wood and histopathology of nematode-infested trees. Nematologica 18:120-124.
23. Miklos, G. L. G. 1985. Localized highly repetitive DNA sequences in vertebrate and invertebrate genomes. Pages 241-321 in: Molecular Evolutionary Genetics. R. J. MacIntyre, ed. Plenum Press, New York.
24. Nickle, W. R., Golden, A. M., Mamiya, Y., and Wergin, W. P. 1981. On the taxonomy and morphology of the pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle 1970. J. Nematol. 13:385-392.
25. Piotte, C., Castagnone-Sereno, P., Bongiovanni, M., Dalmasso, A., and Abad, P. Cloning and characterization of two satellite DNAs in the low C-value genome of the nematode *Meloidogyne* spp. Gene. In press.
26. Piotte, C., Castagnone-Sereno, P., Uijthof, J., Abad, P., Bongiovanni, M., and Dalmasso, A. 1992. Molecular characterization of species and populations of *Meloidogyne* from various geographic origins with repeated-DNA homologous probes. Fund. Appl. Nematol. 15:271-276.
27. Rhüm, W. 1956. Die Nematoden der Ipiden. Parasitol. Schriftenr. 6:1-487.
28. Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
29. Schauer-Blume, M. 1989. Report of the panel on the pine wood nematode. Eur. Mediterr. Plant Prot. Organ. Bull. 89/2095:11-12.
30. Schots, A., Hermsen, T., Shouten, S., Gommers, F. J., and Egberts, E. 1989. Serological differentiation of the potato-cyst nematodes *Globodera pallida* and *G. rostochiensis*: II. Preparation and characterization of species specific monoclonal antibodies. Hybridoma 8:401-413.
31. Skinner, D. M. 1977. Satellite DNAs. Bioscience 27:790-796.
32. Smith, G. E., and Summers, M. D. 1980. The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzylomethyl-paper. Anal. Biochem. 109:123-129.
33. Tarès, S., Abad, P., Bruguier, N., and de Guiran, G. 1992. Identification and evidence for relationships among geographical isolates of *Bursaphelenchus* spp. (pinewood nematode) using homologous DNA probes. Heredity 68:157-164.
34. Tarès, S., Lemontey, J. M., de Guiran, G., and Abad, P. 1993. Cloning and characterization of highly conserved satellite DNA sequence specific for the phytoparasitic nematode *Bursaphelenchus xylophilus*. Gene 129:269-273.
35. Webster, J. M., Anderson, R. V., Baillie, D. L., Beckenbach, K., Curran, J., and Rutherford, T. A. 1990. DNA probes for differentiating isolates of the pine wood nematode species complex. Rev. Nématol. 13:255-263.
36. Wingfield, M. J., Blanchette, A., and Kondo, E. 1983. Comparison of the pine wood nematode, *Bursaphelenchus xylophilus*, from pine and balsam fir. Eur. J. For. Pathol. 13:360-373.
37. Yan, B., and Wang, Q. 1989. Distribution of the pinewood nematode in China and susceptibility of some Chinese and exotic pines to the nematode. Can. J. For. Res. 19:1527-1530.