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Boty, a Long-Terminal-Repeat Retroelement in the Phytopathogenic Fungus *Botrytis cinerea*

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The phytopathogenic fungus *Botrytis cinerea* can infect an extremely wide range of host plants (tomato, grapevine, strawberry, and flax) without apparent specialization. While studying genetic diversity in this fungus, we found an element which is present in multiple copies and dispersed throughout the genome of some of its isolates. DNA sequence analysis revealed that the element contained direct, long-terminal repeats (LTRs) of 596 bp whose features were characteristic of retroviral and retrotransposon LTRs. Within the element, we identified an open reading frame with sequences homologous to the reverse transcriptase and RNase H domains of retroelement *pol* genes. We concluded that the element we had identified was a retroelement and named it Boty. By comparing its open reading frame with sequences from other retroelements, we found that Boty is related to the gypsy family of retrotransposons. Boty was present in numerous strains isolated from grapes and tomatoes but not in isolates from lentils. We propose that Boty-containing and Boty-deficient groups represent two lineages in the population of *B. cinerea*.

Botrytis cinerea is a fungal plant pathogen which shows considerable variation in that it parasitizes well over 200 hosts without apparent specialization. It attacks field and glasshouse vegetables (tomatos, cucumbers, and lettuce), small berry fruits (grapes), ornamentals, bulbs, and corm-producing monocotyledons. Isolates of *B. cinerea* that infect grapes cause a devastating disease known as grey mold. Our objective was to study the nature and extent of genetic variability in *B. cinerea*. Such knowledge can provide clues to the mechanisms of adaptability on different hosts and thus can serve as a basis for effective disease management. Studies employing isozyme analysis and restriction fragment length polymorphism analysis in ribosomic units were inconclusive in defining the nature and extent of genetic variation (unpublished results). Therefore, we decided to analyze the genome of *B. cinerea* pathogens for the presence of repetitive DNA sequences. In other organisms, repeated DNA elements have been shown to accumulate sequence polymorphisms at a high rate, presumably because of genetic drift and rearrangement (6). A dispersed repeated DNA sequence isolated from the filamentous fungus *Cryphonectria parasitica* was shown to be very useful in DNA fingerprinting and in the analysis of the population structure of this chestnut pathogen (18). In *Magnaporthe grisea*, the study of the distribution and amplification of two different dispersed repeated sequences, MGR (8) and Grh (3), allowed the authors to propose a clonal history of host-specific forms of *M. grisea*.

We identified a family of polymorphic dispersed repeated DNA sequences from the genome of *B. cinerea* 397 isolated from tomato plants, and we named it Boty. We then analyzed a number of *B. cinerea* pathogens isolated from different plants cultivated in various regions of France for the presence of Boty.

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MATERIALS AND METHODS

Fungal strains and culture media. We used *B. cinerea* isolates from grapes, tomatoes, flax, lentils, and peas. Their origins are shown in Table 1. All of the isolates were purified by monospore isolation and conserved with paraffin oil at 16°C.

For DNA extraction, we cultured the mycelium in 50 ml of N1 medium (20 g of cristomalt per liter, 20 g of yeast extract per liter, and 5 g of casein hydrolysate per liter) by first flooding 2-week-old agar slant cultures with 10 ml of sterile H_2O, scraping the culture with a sterile loop to dislodge conidia, and finally, decanting the liquid into Roux flasks. The cultures were incubated for 2 days at 20°C before the mycelium was filtered through Miracloth, lyophilized, and ground to a fine powder for DNA extraction.

Bacterial strains and plasmid. *Escherichia coli* DH5α (BRL Life Technologies, Inc., Gaithersburg, Md.) were used for the construction, propagation, and amplification of plasmids as described by Sambrook and coworkers (22). Plasmid pUC19 was used for the cloning of repeated elements.

Isolation and manipulation of DNA. We prepared strain 397 fungal genomic DNA on a large scale from frozen mycelium as described by Daboussi and coworkers (2) for the fungus *Fusarium oxysporum*. A minipreparation of *B. cinerea* genomic DNA was obtained from lyophilized mycelia by the method of Möller et al. (19).

DNA was digested with restriction enzymes, ligated, and electrophoresed by standard procedures. DNA was labeled in vitro with ^{32}P by random priming (Amersham kit), and the probes obtained were used to hybridize DNA transferred on nylon membranes (Hybond N; Amersham, Amersham, United Kingdom) with a vacuum blotting system (Pharmacia, Uppsala, Sweden). All steps of the hybridization experiments were done as recommended by the suppliers.

Construction of minilibraries. The total DNA of strain 397 (50 μg) was digested with *Hin*dIII and electrophoresed on 0.6% agarose gel. Under UV light, four adjacent slices which contained DNA fragments ranging from 1.3 to 8 kb were cut from the gel. Each slice was then separately electroeluted and inserted by ligation into *Hin*dIII-cut pUC19.

The sets of ligation obtained were used to transform *E. coli* DH5α and construct minilibraries. The three sets obtained with fragments of 1.3 to 2.5, 2.5 to 3.5, and 3.5 to 5.5 kb each gave 250 recombinant clones. The set obtained with fragments of 5.5 to 8 kb gave 120 recombinant clones. All 870 clones were tested for the presence of repeated sequences.

Colony blot procedure. To screen *B. cinerea* strains for the presence or absence of the repeated sequences, we used the colony blot procedure developed for *Neurospora crassa* (14), which we modified by directly laying a drop of a spore suspension (10^{10} spores per ml) on nylon membranes. The membranes were then treated as described by the author. *E. coli* colony blot was performed by standard procedures (22).

DNA sequencing. DNA sequences were determined by the dideoxyribonucleotide chain termination method (23) using modified T7 polymerase (Pharmacia kit) (27). We used either universal primers or primers deduced from sequencing and synthesized on an Applied Biosystems apparatus model 381 A.

The Boty sequence was compared with those of various retroid elements.

TABLE 1. Strains of *B. cinerea* used in this study[a]

Strain no.	Host	Location (department no.)	No. of Boty-containing clones/ total no. of clones
73 to 88, 364, 365, 366	Grapevine	Bordeaux (33)	16/19
94 to 131	Grapevine	St. Emilion (33)	35/38
142, 143, 144, 146, 149, 153, 154, 155, 156	Grapevine	Pyrenées orientales (66)	9/9
580 to 588, 614, 615	Grapevine	Vaucluse (84)	10/10
217	Grapevine	Tours (37)	1/1
367, 368	Grapevine	Marne (51)	2/2
396 to 399, 409, 402 to 404, 406, 607	Tomato	Bouches du Rhone (13)	9/10
401, 407, 408	Tomato	Pyrenées orientales (66)	3/3
610	Tomato	Yvelines (78)	1/1
612	Tomato	Vaucluse (84)	1/1
613, 410	Tomato	Finistère (29)	2/2
421, 422, 424, 434, 435, 441, 449, 455, 456, 459, 464, 470, 475, 477	Lentil	Cher (18)	0/14
487, 430, 481, 482, 487	Flax	Unknown	0/5
425, 426, 427, 429	Pea	Calvados (14)	0/4

[a] Strains of *B. cinerea* isolated from grapevines and tomatoes in Vaucluse were received from P. Nicot, Institut National de la Recherche Agronomique, Avignon, France. Strains 364, 365, and 366 were received from G. Bompeix, University of Paris VII, Paris, France, and strains 367 and 368 were from P. Leroux, Institut National de la Recherche Agronomique, Versailles, France. Strains isolated from flax, peas, and lentils were received from GEVES, Angers, France. The other strains, collected in 1992, were from our collection. Locations are specified by the name of the department in France and by its number (in parentheses).

Amino acid sequences and features of retrotransposons were obtained from GenBank and from references. Multiple alignments were made with the CLUSTAL program (11).

Nucleotide sequence accession number. The DNA sequences of Boty's long-terminal repeat (LTR) and *pol* genes have been entered in the EMBL databank and assigned the accession numbers X81790 and X81791, respectively.

RESULTS

Isolation of a repeated DNA sequence from *B. cinerea*. To isolate repeated DNA in the genome of *B. cinerea* 397, we used the hybridization strategy developed for *M. grisea* (8). We identified recombinant clones containing repeated DNA sequences in a *Hin*dIII minilibrary by use of total genomic DNA as a hybridization probe. These clones gave an intense signal with the genomic DNA probe. Twenty-three of the 870 recombinant clones hybridized intensely to total *Hin*dIII-digested genomic DNA.

We successively extracted and tested each of the recombinant plasmids for the presence of a dispersed repeated DNA sequence by probing a Southern blot of the genomic DNA of strain 397 digested by *Hin*dIII and *Eco*RI. Two of the plasmids, pBH6 and pBH14, which yielded obvious repeated banding patterns (Fig. 1) were selected for further study. Figure 2a shows their restriction maps. They both contained a 500-bp *Pst*I-*Bam*HI-*Xba*I fragment which cross-hybridized in stringent conditions (data not shown). We therefore postulated that pBH6 and pBH14 were part of the same repeated element having an LTR. We confirmed this hypothesis by probing *Pst*I- and *Xba*I-digested genomic DNA successively by pBH6 and pBH14. Both plasmids hybridized with an intense signal to the same 6-kb *Pst*I and 6-kb *Xba*I fragments (data not shown), both of which probably contain the full-length copies of the repeated element deprived of one of the two *Pst*I-*Xba*I terminal fragments. To characterize this element, we decided to clone the 6-kb *Pst*I-digested DNA fragments. They were first electroeluted from an agarose gel and then cloned into pUC19. We screened the recombinant clones for the presence of repeated sequences with an insert of pBH6 as a probe. Five positive clones were selected. The physical map of one of these clones, pBP16, was drawn, and we located pBH6 and pBH14 on the 6-kb *Pst*I fragment (Fig. 2b). The copies of the element were polymorphic, considering the *Bam*HI and *Eco*RI sites on pBH6, pBH14, and pBP16 (Fig. 2). When *Eco*RI-digested DNA of strain 397 was probed with pBH6 or pBH14, two bands of 1.1 and 1.4 kb or three bands of 2.1, 2.4, and 3.3 kb, respectively, gave an intense signal (Fig. 1). These bands may correspond to internal polymorphic *Eco*RI fragments. The former were detected only when the subclone p6E11 from pBH6 (Fig. 2) was used as a probe, while the latter were detected when the probe was the subclone p14E13 from pBH14 (data not shown). This result indicates that the repeated element we have cloned contains polymorphic sequences and that at least three different forms coexist in the genome of strain 397.

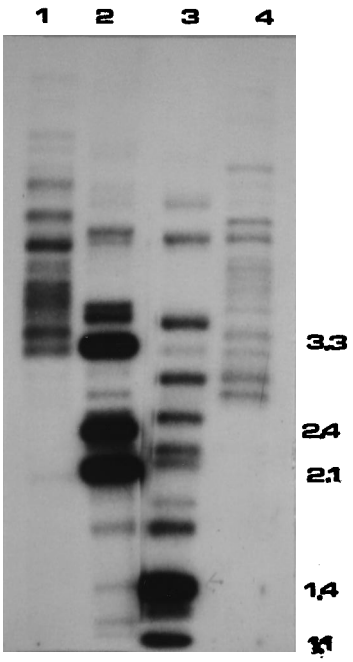

FIG. 1. Southern blot analysis of total DNA of *B. cinerea* 397 digested by *Hin*dIII (lanes 1 and 4) or *Eco*RI (lanes 2 and 3) and probed with ^{32}P-labeled pBH6 (lanes 3 and 4) or pBH14 (lanes 1 and 2). Sizes of DNA fragments are indicated in kilobases.

The size and physical organization of this repeated element led us to postulate that it is a retrotransposon with an LTR. We named it Boty.

Boty is an LTR-class retroelement. DNA sequencing of the *Pst*I-*Xba*I region in pBH6 and pBH14 confirmed that the element terminated in 596-bp perfect direct repeats (Fig. 3) which were strictly identical in the two plasmids. The direct repeats contained the 5′- and 3′-terminal sequences (5′TG...CA3′) and perfect short inverted terminal repeats of 7 bp that are hallmarks of retroviral LTRs and of most retrotransposon ones (1, 29). The terminal repeat in pBP16 (lacking the first 77 bp 5′

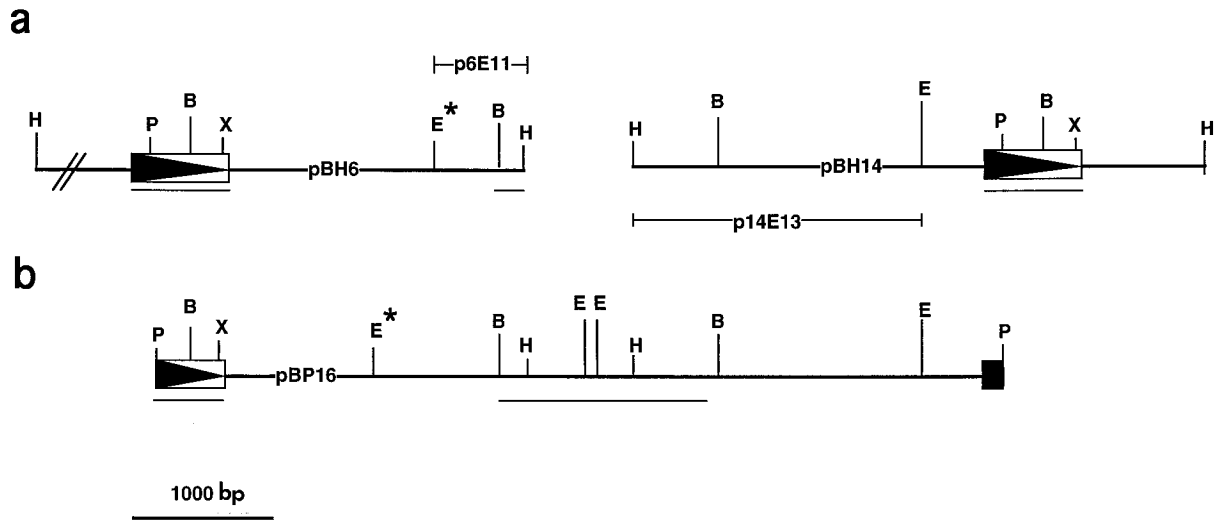

FIG. 2. Restriction maps of *B. cinerea* clones containing repeated sequences specific to *B. cinerea* 397. Abbreviations for restriction sites: H, *Hin*dIII; E, *Eco*RI; P, *Pst*I; B, *Bam*HI; X, *Xba*I. (a) Restriction maps of pBH6 and pBH14. The *Pst*I-*Bam*HI-*Xba*I region present in pBH6 and pBH14 cross hybridized. This was detected by Southern blot analysis of pBH6 digested with different restriction enzymes probed with pBH14. p6E11 and p14E12 are subclones obtained by cloning the appropriate *Hin*dIII-*Eco*RI fragment from pBH6 and pBH14, respectively, into pUC19. (b) Restriction map of pBP16. E* corresponds to *Eco*RI sites that are not in the same location in pBH6 and pBP16. The regions containing LTR sequences in each clone are illustrated by black arrows. The DNA regions which were sequenced are underlined in the physical maps of pBH6, pBH14, and pBP16.

upstream of the cloning site *Pst*I) was identical to those of pBH6 and pBH14. Immediately 3′ to the 5′ LTR is the presumed primer binding site for first-strand reverse transcription (1). This site, characterized by a highly conserved 5′ TGG in most *Drosophila* retransposons, could not be identified in Boty. This sequence was found neither in Grh of *M. grisea* nor in Cft1 of *Cladosporium fulvum*. However, a polypurine-rich sequence that could correspond to the primer binding site for plus-strand DNA synthesis was located immediately upstream of Boty's 3′ LTR in pBH14. This sequence is 5′-AGGCTAAGAAGGG GATAG-3′.

By first sequencing 200 bp from the right *Hin*dIII site of pBH6, we identified a similarity with the N-terminal extremity of the putative reverse transcriptase (RT) of Cft1, a retrotransposon of the gypsy branch isolated from *C. fulvum* (17). We then decided to sequence in pBP16 all of the region coding to the RT starting near the internal *Bam*HI site (Fig. 2b). Figure 4 shows the DNA sequence of a 1.2-kb internal region of pBP16. Computer analysis identified only one open reading frame. Two regions of the open reading frame offer similarities with the polyprotein sequence encoded by *pol* genes from retrotransposons of the gypsy family (12). A 180-amino-acid region of Boty's open reading frame contains the seven domains characteristic of RT sequences with the highly conserved YXDD sequence (30, 31). Figure 5 shows the alignment of the amino acid sequence of Boty's RT with seven elements of the gypsy group of retrotransposons. The amino acid sequence of Boty was compared pairwise with those in other retroelements (Table 2). The percentage of identity ranged from 34 to 53%, which is significant for related sequences (4). The sequence analysis of *pol* clearly demonstrates that Boty is most closely related to (and thus is a member of) the gypsy class of the retrotransposon family. Downstream of the putative RT are sequences homologous to the RNase H domains of gypsy-related retroelements. Figure 6 presents the best alignment of Boty's RNase H amino acid domain (from nucleotides 693 to 1224 in Fig. 4) with RNase H domains of fungi retroelements. The percentage of identity calculated from the first 162 amino acids downstream of the RT is given in Table 2. It is lower than that calculated from the RT. Doolittle and coworkers (5) estimate that RNase sequences change at a faster rate than those of RT, but they show that phylogeny determined from the alignment of RNase H sequences is consistent with the gypsy group defined previously. As was shown for the RT sequence, Boty is more closely related to Cft1 than to Grh or Foret 1.

Distribution of Boty in populations of *B. cinerea*. By preliminary restriction fragment length polymorphism analysis using pBH6 as a probe and under stringent conditions, we could detect Boty sequences in strains isolated from tomatoes and grapes but not in isolates from lentils, peas, or flax (data not shown). Then, by use of the dot blot procedure (14), we looked

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AAAGTTTTGATTGAAGAAGAAATTGAAATGAAATAAGTGAATGATTTACCC
CTTACTTCCCCTCCCCTTTAAACTTTCTGTTACGACGATTAGTAACAGGCT
GTAGAATCACCAACGTATAGGCTATAATGGTATTATAGGCCTCAGCGATTC
AGCTGCAGTATACTGGGGGACACTAGGCACCCAAGGAAAGCCTCAGGCATG
TATATAGTATTAGTCATAGGATATCCTAGAAACGTAGGACAATAGGTCCTA
GGAAACACCGAACATAACTTTGCAAACTTTTCGCGAAGTTATATTAGTAAT
GTCCCAGGGGATTGGCCCTAGGATAAAAGATAAGCTAGGACACGGGAAGAC
ACGGGAACCGGTATCACGTGATGGGCCGTCCCATCAATCCCACCGATTATT
ATCAATGGGATCATGGGGATCCATCACTCCCACCACTTCCGGTGAGTGGGA
TCGATGGGACAACAGAGTACGAAGGTCTATATAAGGGAATGGGTTTCCTTA
TATGTAGAGCTTCGTGCTCAAGAACAATCATTAGTTTCATTACTATAGTTA
CGAGAATTGCAATCAGTTACAACCTTATTGAATCCCTACTTGAAGTCTAGT
CTAAACCACCTCGAGAGATCTCTAGACACTTCCACGTGACCCTAGAGGCAG
CTCCCGTAACACTTTGAGCACCCTTTCTGGCCAAGTACCGATTCGATAACC
CACCGCTGAAATATGGCAACCAGAAATACCGCCACAGGTCAGTCTACCGG
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FIG. 3. Nucleotide sequences of 596-bp LTRs present in pBH6. The 7-bp terminal inverted repeats with the underlined 5′ TB...CA 3′ nucleotides characteristic of retroviral LTR termini are in bold type. The 5′ *Pst*I site and the 3′ *Xba*I site are underlined.

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1/1                                                 31/11
TTT CAA GAC CAG GGT CCT GAG GCT TTA CCG GAA CAC AAG CCA TGG GAT CAT GAG ATA ATA
F   Q   D   Q   G   P   E   A   L   P   E   H   K   P   W   D   H   E   I   I
61/21                                               91/31
TTG GAG GAA GGC AAG ATG CCT GTG CAC ACC CCA ATT TAT TCA ATG TCA GCC GAT GAG TTA
L   E   E   G   K   M   P   V   H   T   P   I   Y   S   M   S   A   D   E   L
121/41                                              151/51
AAA AGG CTC AGA GAA TAC ATC GAC GAC AAT TTA GCC AAG GGA TGG ATC AGG GAA TCC GCG
K   R   L   R   E   Y   I   D   D   N   L   A   K   G   W   I   R   E   S   A
181/61                                              211/71
TCC CAA GTG GCC AGT CCA ACT ATG TGG GTA CCC AAG AAG GAT GGA CCC GAT AGA CTA GTT
S   Q   V   A   S   P   T   M   W   V   P   K   K   D   G   P   D   R   L   V
241/81                                              271/91
GTA GAC TAT AGA AAG CTT AAC ACA CTC ACT AAG AAG GAT CGA TAT CCA CTT CCA TTA GCT
V   D   Y   R   K   L   N   T   L   T   K   K   D   R   Y   P   L   P   L   A
301/101                                             331/111
ACG GAA TTA AGA GAT CGG TTA GGC GGA CGT ACG ATA TTT ACC AAG ATG GAC CTA CGT AAT
T   E   L   R   D   R   L   G   G   R   T   I   F   T   K   M   D   L   R   N
361/121                                             391/131
GGT TAC CAC TTG ATC AGA ATG AAG GAA GGC GAA GAA TGG AAG ACC GCT TTC AAA ACA AGA
G   Y   H   L   I   R   M   K   E   G   E   E   W   K   T   A   F   K   T   R
421/141                                             451/151
TAC GGG CTA TAC GAG TAC CAA GTT ATG CCG TTC GGG CTA ACC AAC GCA CCA GCG ACT TTC
Y   G   L   Y   E   Y   Q   V   M   P   F   G   L   T   N   A   P   A   T   F
481/161                                             511/171
ATG AGG CTT ATG AAC AAT GTG TTG TCA CAA TAC TTG GAT ACT TGC TGT ATA TGC TAC TTG
M   R   L   M   N   N   V   L   S   Q   Y   L   D   T   C   C   I   C   Y   L
541/181                                             571/191
GAC GAC ATC CTA GTA TAT TCA AAC AAC AAG GTT CAA CAC ATT AAG GAC GTT AGC AAC ATC
D   D   I   L   V   Y   S   N   N   K   V   Q   H   I   K   D   V   S   N   I
601/201                                             631/211
CTC GAA AGC CTA TCC AAG GCA GAC TTG CTG TGC AAA CCA AGC AAA TGC GAA TTC CAT GTC
L   E   S   L   S   K   A   D   L   L   C   K   P   S   K   C   E   F   H   V
661/221                                             691/231
ACA GAG ACA GAA TTC TTG GGA TTC ACC GTA TCA AGC CAA GGG CTC AAG ATG AGC AAA GAC
T   E   T   E   F   L   G   F   T   V   S   S   Q   G   L   K   M   S   K   D
721/241                                             751/251
AAG GTT AAG GCA GTG CTC GAA TGG AAG CAG CCA ACC ACA ATC AAG GAG GTA CAA TCC TTT
K   V   K   A   V   L   E   W   K   Q   P   T   T   I   K   E   V   Q   S   F
781/261                                             811/271
CTA GGG TTC GTC AAC TTC TAC AGA AGA TTT ATC AAG GGT TAT TCA GGG ATT ACT ACA CCC
L   G   F   V   N   F   Y   R   R   F   I   K   G   Y   S   G   I   T   T   P
841/281                                             871/291
TTG ACC ACG TTA ACC AGA AAA GAT CAA GGA AGC TTC GAA TGG ACT GCC AAA GCA CAG GAG
L   T   T   L   T   R   K   D   Q   G   S   F   E   W   T   A   K   A   Q   E
901/301                                             931/311
TCA TTC GAT ACG CTC AAA CAA GCA GTG GCA GAA GAG CCA ATA CTA TTG ACT TTT GAC CCA
S   F   D   T   L   K   Q   A   V   A   E   E   P   I   L   L   T   F   D   P
961/321                                             991/331
GAG AAA GAA ATC ATA GTG GAG ACG GAC TCC TCG GAT TTC GCT ATA GGA GCA GTT CTG AGC
E   K   E   I   I   V   E   T   D   S   S   D   F   A   I   G   A   V   L   S
1021/341                                            1051/351
CAA CCG GGC CAG AAT GGA AAA TAC CAG CCA ATC GCA TTC TAT TCC CGA AAA CTA TCA CCA
Q   P   G   Q   N   G   K   Y   Q   P   I   A   F   Y   S   R   K   L   S   P
1081/361                                            1111/371
GCT GAG TTG AAT TAC GAG ATA TAT GAC AAA GAA TTA CTG GCG ATA GTC GAT GCA TTT AGA
A   E   L   N   Y   E   I   Y   D   K   E   L   L   A   I   V   D   A   F   R
1141/381                                            1171/391
GAA TGG CGA GTA TAT TTG GAA GGT CGA TAC ACA GTA CAG GTG TAC ACA GAC TTG GTT ACT
E   W   R   V   Y   L   E   G   R   Y   T   V   Q   V   Y   T   D   L   V   T
1201/401
TCA CCA CAA CGA GCG TAC CGG CGC
S   P   Q   R   A   Y   R   R
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FIG. 4. DNA sequence of 1,224 bp of the internal region of Boty present in pBP16. The amino acid sequence in the one-letter amino acid code is shown above the proposed reading frame. Regions with similarity with the RT and RNase H domains of LTR retroelement *pol* polyproteins are from nucleotides 153 to 692 and 693 to 1224, respectively. The highly conserved sequence YXDD is indicated in bold type.

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                ----------1----------        ---------2------
Del             L-NKGFIRGSTSPWGAHVLFDPKKDDSK------RMCIDY*KLNSVTVKN
Gypsy           LKDG-IIRPSRSPYNSPTWVVDKKGTDAFGNPNKRLVIDFRKLNEKTIPD
Ty3             LDNK-FIVPSKSPCSSPVVLVPKKD-GTF-----RLCVDYRTLNKATISD
Tf1             LKS-GIIRESKAINACPVMFVPKKEGTL------RMVVDYKPLNKYVKPN
grh             MMDKGWIRASSSSAAAPVLMVRKASGGW------RLCVDYRALNSITMQD
Cft1            L-AKGWIRRSTSSAGTPCMFVPKANGKL------RLVQDYRKLNEITIKN
Foret1          LAKRN-IRISHYSAGFLVIFIPKRIKKL------*LVIDYRKTNALIIKD
Boty            L-AKGWIRESASQVASPTMWVPKKDGPD------RLVVDYRKLNTLTKKD
                      ** *           *            *   ***  *

                ----------+----------3------------------+---
Del             KYPLPRIDDLFDQLNGA*YFSKIDLRFRYHQLRIRA*DIPKTAFRTRYGH
Gypsy           RYPMTSIPMILANLGKAKFFTTLDLKSGYHQIYLAEHDREKTSFSVNGGK
Ty3             PFPLPRIDNLLSRIGNAQIFTTLDLHSGYHQIPMEPKDRYKTAFVTPSGK
Tf1             IYPLPLIEQLLAKIQGSTIFTKLD-KSAYHLIRVRKGDEHKLAFRCPRGV
grh             RYPLPLIKETIRSLTGARWFTKVDVRAAFHKLRIAEGDEHLTAFRTRFGL
Ct1             RYPLPNIEEAQDRLTGSDWYTKIDLRDAFYAIRMAEGEEWKTAFRTKFRL
Foret1          RTLLLLITGLKDRYRQIDLFTALDLKGAYNLICIKEGDKWKTAFRTKFRL
Boty            RYPLPLATELRDRLGGRTIFTKMDLRNGYHLIRMKEGEEWKTAFKTRYGL
                *  *                 *  *           **    *** *   *

                ----------------4----------------+-------5---------
Del             YEFLVMPFGLTNVPTAFMNLMNRVFREYLDKFIVVFVDYVLIYSRTQ-KD
Gypsy           YEFCRLPFGLRNASSIFQRALDDVLREQIGKICYVYVDDVIIFSENE-SD
Ty3             YEYTVMPFGLVNAPSTFARYMADTFRDL--RFVNVYLDDILIFSESP-EE
Tf1             FEYLVMPYGISTAPAHFQYFINTILGEAKESHVVCYMDDILIHSKSE-SE
Grh             FEWLVCPFGLAGAPATFQRYVNGVLGDTLGDYASAYLDDILIYSSGSKSD
Cft1            YEFLVMPMGLTNAPASCQDLVNETLRDLLDVCVVAYMDDILVYTKGSLQE
Foret1          FKYLVMLFGLTNASVCLQIINNILLIVYLDVFIVSYLDDILIFLDNK-EE
Boty            YEYQVMPFGLTNAPATFMRLMNNVLSQYLDTCCICYLDDILVYSNNKVQ-
                    *   **  *         *  *   *      * ****

                -------------6-----------+-----7------
Del             HEHHLRISLQLLRNNQLYAKLSKCEFWMEKVKFLGHVV
Gypsy           HVRHIDTVLKCLIDANMRVSQEKTRFFKESVEYLGFIV
Ty3             HWKHLDTVLERLKNENLIVKKKKCKFASEETEFLGYSI
Tf1             HVKHVKDVLQKLKNANLIINQAKCEFHQSQVKFIGYHI
Grh             HWSKVTRVLDKLAAAGLNLDLDKSAFAVKEVKYLGFIV
Cft1            HTKQVQDVFERLTKSGFKTAPEKCEFHKKEVKFLGFII
Foret1          HYEYIYKVLKAL*DTNLLVKPEKSYFYVKEVDFLGYTI
Boty            HIKDVSNILESLSKADLLCKPSKCEFHVTETEFLGFTV
                *         *          *  *    *   **
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FIG. 5. Alignment of inferred Boty RT amino acid sequence with other retroid elements shown in N- to C-terminus order. The single-letter code for amino acids is used. Other sequences are of Del (24) from *Lilium henryi*, gypsy (16) from *Drosophila melanogaster*, Ty3 (10) from *Saccharomyces cerevisiae*, Tf1 (15) from *Schizosaccharomyces pombe*, Grh (3) from *M. grisea*, Cft1 (17) from *C. fulvum*, and Foret 1 (13) from *F. oxysporum*. Numbers 1 to 7 refer to domains identified by Xiong and Eickbush (30). An asterisk in the sequence indicates a stop codon. Amino acids in bold type are invariant among presented RTs. Amino acids common to fungus RTs are indicated below the Boty sequence with an asterisk.

for the presence or absence of Boty in 119 isolates collected either from grapes, tomatoes, lentils, peas, or flax in different regions of France (Table 1).

Boty was present in 16 of the 17 isolates collected from tomatoes in five regions of France and in 73 of the 79 isolates collected from grapes in six regions of France. None of the 5 isolates from flax, the 4 isolates from peas, or the 14 isolates from lentils contained Boty sequences.

DNA fingerprinting of *B. cinerea* vineyard isolates. Southern blots of *Eco*RI-digested genomic DNA of 11 isolates of *B. cinerea* collected in two vineyards in the St. Emilion region in France were probed with pBH14. Nine distinctive patterns were observed (Fig. 7). Strains 110 and 111 isolated from the same vine stock displayed different patterns. Strain 111 displayed the same pattern as strain 112, which was collected in the same vineyard and on the same type of vine (Cabernet Sauvignon). Strains 118 and 121, which had identical patterns, were isolated in a second St. Emilion vineyard and in the same vine stock (Merlot). In each grape isolate, the same four *Eco*RI bands (of 2.1, 2.4, 3.2, and 3.3 kb) gave an intense signal. These bands may correspond to a variable internal *Eco*RI fragment of the different copies of Boty in the same genome. This result indicates that the same forms of Boty coexist in each strain.

DISCUSSION

We have identified, in the genome of *B. cinerea*, a retroelement related to the gypsy class of LTR-containing transposons. The size and the presence of a single, uninterrupted reading frame encoding putative RT and RNase proteins and the identity of three LTRs cloned at different genomic locations suggest that at least some copies of the retroelement are intact, functional, and have recently transposed. Further sequencing and characterization of Boty are in progress to examine its overall protein coding capacity. Boty is presumed to give rise to repeated sequences and may cause important evolutionary variations. Gypsy-class retroelements have been identified previously in insects (16), yeasts (10, 15), plants (24), and other

TABLE 2. Percent identity of amino acid sequences of Boty RT and RNase regions with those in other retroelements[a]

Element	% Amino acid identity of:	
	RT	RNase
Gypsy	34	ND[b]
Tf1	41	ND
Ty3	43	ND
Del	44	ND
Foret 1	43	30
Grh	45	36
Cft1	53	45

[a] The covered domains are 180 amino acids for RT and 162 amino acids for RNase.
[b] ND, not determined.

filamentous fungi (3, 13, 17). Springer and Britten (25) defined clades on the basis of the phylogenetic relationships of RT and RNase H sequences in the gypsy branch of retrotransposons. Their analysis included only one retrotransposon isolated from fungi. Our analysis of the phylogenetic relationships (using the PAUP computer program [26]) of the entire RT sequences, including Boty, Cft1, Foret 1, and Grh from fungi, Del from lily, Ty3 and Tf1 from yeast, and gypsy from Drosophile did not allow us to define clades that could be supported by bootstrapping. The presence of these related elements in widely different organisms can be explained by the fact that they may result from horizontal transmissions in recent evolutionary time. Indicative of horizontal transfer is the grouping reported in *Arabidopsis thaliana*, where LTR elements closely related to insect ones coexist with other LTR elements in the same plant species, suggesting a recent extrachromosomal origin for the first set of elements (7). Interestingly, Boty isolated from tomato plants is more closely related to Cft1, another tomato pathogen. It would be interesting to know if tomato plants contain LTR elements related to Boty and Cft1, which would suggest that a horizontal transfer took place between the plants and the pathogens.

Boty sequences were present in numerous grape and tomato pathogens from diverse locations in France, suggesting that these pathogens are descendants of a small ancestral population. The wide distribution of grapes and tomato plants in France appears to have provided niches for the growth and dispersal of these ancestral pathogen populations. Nevertheless, few tomato or grape pathogens lack Boty sequences, suggesting that the retrotransposon invaded the ancestral genome after pathogenicity toward grapes and tomatoes evolved.

```
Cft1    STTGITIDPAKTQSIREWPEPKTVKDVQSFLGLANYNRKFIKDYSKTAAPMT------
Grh     AGEGVQADPEKIKAIRDWEAPTRLRGLRGFLGFANFYRDFIDGYSTLTAPLLALTK--
Foret   TLGEIRIKQKKISVVADYPLLIIIKEVqSFLGFTNYYrRFIRDFSKIINPLTKLIK--
Boty    SSQGLKMSKDKVKAVLEWKQPTTIKEVQSFLGFVNFYRRFIKGYSGITTPLTTLTR--

Cft1    ---MLTAKDVNWK-WGKEQTEAFKRLKEQCASAPTLRLFDGS-KEVHIETDASDMAIGA
Grh     --------K-GTPFRWTEELEGAFEALKEAFLQAPILAQWDDA-KDTRMETDCSGAALGG
Foret   --------KDRKFI-wKkDAQEAFKQL*eAILSEPILVIFALIRKeNSKPTY*TSY*EDK
Boty    -------KDQGSFEWTAKAQESFDTLKQAVAEEPILLTFDPE-KEIIVETDSSDFAIGA

Cft1    CLTQTHDGKRH-PVAYYSAKMTTAEQ-NYDIHDKE--LLAIVAAMQHWR--VYVEGPPKL
Grh     CLSGKGTDGLWRPVAFHSAKLTDAQR-NYTIHDKE--LLA-VIACLKAWDAELRSVRRPF
Foret   -LDNAITKASYTLLHSTCISI*TRTyLPYLQ*GVSAGLLTTLRNLDTTL*EVYTKSKSIL
Boty    VLSQPGQNQKYQPIAFYSRKLSPAE-LNYEIYDKE--LLAIVDAFREWR--VYLEGRYTV

Cft1    TILSDHKNLTYFTTTK
Grh     LILTDHKALEYFSKPR
Foret   TIRTFILYYDLRTLTK
Boty    QVYTDLVTSPQRAYRR
```

FIG. 6. Alignment of the inferred Boty RNase amino acid sequence with those of other retroid elements isolated from fungi shown in N- to C-terminus order. The single-letter code for amino acids is used. Amino acids in bold type are invariant among presented RTs. Lowercase letters are used in the sequence of Foret 1 when a single mutation allows the replacement of a stop codon by the amino acid present in the Cft1 sequence. An asterisk indicates a stop codon.

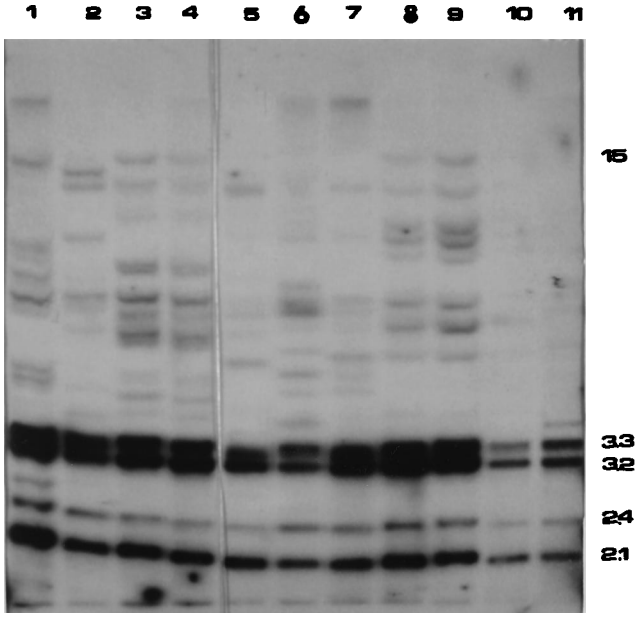

FIG. 7. Southern blot analysis of *Eco*RI-digested DNA from strains of *B. cinerea* collected from grapes in the same region of St. Emilion in France probed with pBH14. Strains 109, 110, 111, 112, 115, and 116 (lanes 1 to 6, respectively) were collected in the same vineyard, Chateau Dassault Peltan, and strains 117, 118, 121, 119, and 120 were from a second vineyard, Chateau Angelus. Bands with an intense signal correspond to polymorphic *Eco*RI internal fragments of Boty. Sizes of DNA fragments are indicated in kilobases.

There is sufficient polymorphism in sequences homologous to Boty to demonstrate by Southern blot analysis distinctive patterns in the DNA of many individual grape pathogens isolated from a proximal location. Our results suggest that Boty probes can be suitable for DNA fingerprinting of *B. cinerea* isolates for detailed epidemiological studies. Restriction fragment length polymorphism analysis using Boty probes could also help determine the extent of the diversity and variation in grape isolates from various grey mold areas. In contrast to the high degree of polymorphism detected by Boty probes, very little polymorphism was observed when the restriction fragment length polymorphism analysis used ribosomic genes of *B. cinerea* (unpublished results). It is possible that the length of time over which *B. cinerea* has evolved has been too short to accumulate variations in ribosomic units. On the contrary, in recent times, Boty sequences (i) may have transposed and/or (ii) may have been subjected to recombination events between the copies of the elements, both of which lead to distinctive hybridization patterns. We presume that the sequence variation which we observed in the internal Boty DNA sequence (polymorphic *Eco*RI fragments) arose from single-base-pair changes during the reverse transcription and/or from recombination events between the copies of the elements.

We suggest that the absence of Boty in isolates from lentils correlates with the genetic isolation and independent evolution of these pathogens in the population of *B. cinerea*. This hypothesis was supported by the hybridization pattern of total repeated DNA of genomes which were completely different in lentil isolates and in grape or tomato isolates (unpublished results). Nevertheless, no phenotypic differences (growth, sporulating capacity, or mating ability) were observed between these two groups of pathogens. Broader sampling of the Boty-

containing and Boty-deficient groups will be needed to determine if the two phyla coexist or are strictly geographically limited. It seems that pathogens from peas and flax belong to the Boty-deficient group, but more isolates must be screened before a final conclusion can be reached. Experiments to compare the pathogenic traits of isolates from these two phyla on different host plants are in progress.

Finally, Boty could be used potentially to enhance genetic and molecular approaches to study fungus-plant interactions in *B. cinerea*. Repeated DNA sequences in *M. grisea* have proven useful for genetic mapping. The MGR sequences common to rice pathogens have been used to map pathogenicity genes (9, 20, 28), to construct a genetic map, and to develop marked strains for mapping new mutations (21). Given the distribution of Boty in fertile laboratory strains, it is obvious that it could be used for similar purposes.

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