Virology 417 (2011) 248–252

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Rapid Communication

Boty-like retrotransposons in the filamentous fungus *Botrytis cinerea* contain the additional antisense gene *brtn*

Ming Zhao [a,b], Jin Y. Zhou [a], Zhi D. Li [a], Wei W. Song [a], Tao Gong [a], Hong Tan [a,*]

[a] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China
[b] College of Pu-erh Tea, Yunnan Agricultural University, Kunming, 650201, PR China

ARTICLE INFO

Article history:
Received 25 April 2011
Accepted 21 June 2011
Available online 29 July 2011

Keywords:
Botrytis cinerea
Data mining
Long-terminal repeat
Retrotransposons
Sclerotinia sclerotiorum

ABSTRACT

Long-terminal repeat (LTR) retrotransposons typically contain *gag*, *pol*, or *gag–pol*, and in some case *env* genes. In this work, we used data mining of the *Botrytis cinerea* genomic sequence and a molecular approach to identify *Boty*-like LTR retrotransposons in *B. cinerea* containing an antisense gene (*brtn*) between *pol* and the 3′-LTR. Reverse transcriptase PCR (RT-PCR) revealed that some *brtn*-like genes could be expressed, at least in *B. cinerea* T4. We conducted BLAST comparisons and conserved-domain analysis, but the function of putative *BRTN* is presently unknown. *Boty-like* LTR retrotransposons in *Sclerotinia sclerotiorum*, called *ScscLRET* and containing *brtn* homologs at positions similar to *brtn*, were detected by homology searches and data mining of the *S. sclerotiorum* 1980 genomic sequence. Thus, this study demonstrated that some fungal LTR retrotransposons contain additional antisense genes.

Introduction

Long-terminal repeat (LTR) retrotransposons are an order of mobile genetic elements that transpose via RNA intermediates. They typically consist of *gag*, *pol*, or *gag–pol* genes, in some cases with *env* genes, flanked by an LTR at each end. The *gag* gene encodes structural proteins of the virus particle. The *pol* gene encodes a polyprotein consisting of an aspartic proteinase (AP), a reverse transcriptase (RT), an RNase H (RH), and an integrase (INT) (Wicker et al., 2007). The *env* gene encodes a protein similar to the envelope protein of infectious viral particles (Kim et al., 1994; Vicient et al., 2001). Occasionally, LTR retrotransposons contain additional domains or open reading frames (ORFs). For example, the chromoviruses contain additional dUTPases, as identified in the fungus *Phanerochaete chrysosporium* (Novikova and Blinov, 2008). *Retand-2* in *Silene latifolia* contains two additional ORFs transcribed in the antisense orientation, located between *pol* and the 3′-LTR (Kejnovsky et al., 2006). *RIRE2* of rice and *Grande1* of maize have small ORFs of unknown function in the antisense orientation, downstream of *gag–pol* (Martíinez-Izquierdo et al., 1997; Ohtsubo et al., 1999). *RIRE3*, *RIRE8A* and *RIRE8B* of rice each has an extra ORF of unknown function located upstream of the *gag–pol* region (Kumekawa et al., 1999). A distinct group of Ty3/gypsy retrotransposons (Ogre elements) contain an extra ORF1 encoding a protein of unknown function located upstream of the gag gene. These were first discovered in legume plants, and found to occur in three families of dicot plants (Leguminosae, Solanaceae and Salicaceae) (Macas and Neumann, 2007; Neumann et al., 2003, 2006). *REM1* in the green alga *Chlamydomonas reinhardtii* contains a regulatory module (ORF3p) transcribed in the antisense orientation to the polyprotein, and located upstream of the *gag* gene, that contains a PHD-finger and chromodomains (Pérez-Alegre et al., 2005). The *Bs1* retrotransposon of maize has a transduced cellular gene sequence encoding an ATPase (Bureau et al., 1994; Jin and Bennetzen, 1994).

The LTR retrotransposon *Boty* was identified in *Botrytis cinerea* (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) (Diolez et al., 1995), although only partial *Boty* sequences were reported. The 4.5X whole-genome shotgun sequence (WGS) of *B. cinerea* strain B05.10 is now publicly available (http://www.broad.mit.edu/annotation/genome/botrytis_cinerea.2/Home.html) (Billault et al., 2006). In this work we used data mining and molecular approaches to discover that *Boty*-like retrotransposons contain a potential additional antisense gene of unknown function. This gene, named *brtn*, is located between *pol* and the 3′-LTR, and can be expressed. We also identified a novel LTR retrotransposon that we named *ScscLRET* in *Sclerotinia sclerotiorum*. It contains a *brtn* homolog at a position similar to *brtn* in *Boty*.

Results and discussion

Boty-like elements in the B. cinerea B05.10 genome

Using the *Boty* LTR (GenBank Accession no: X81790) as a query, 60 *Boty-like* LTRs were identified in the *B. cinerea* B05.10 genome (Supplementary material Table 1). The *Boty* LTR shared 37.7–99.7% nucleotide identity to the LTRs identified in this work. Structurally, most

* Corresponding author.
E-mail address: abath@cib.ac.cn (H. Tan).

0042-6822/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2011.06.020

of the LTRs had short inverted terminal repeats of 7 bp (TGTTACG…CGTAACA)/6 bp (TGTTAC…GTAACA)/5 bp (TGTTA…TAACA).

Using the internal *pol* sequence of *Boty* (GenBank Accession no: X81791) as a query, 7 full-length elements and 16 fragmented elements were identified in the *B. cinerea* B05.10 genome (Supplementary material Table 1). *Boty* shared 85.5–96.6% nucleotide identity to the elements found in this work, and the "RT_LTR" domains (cd01647) of *Boty* shared 75.4–99.3% amino acid identity to the RT-LTR domain elements we found. Lin and Levin reported that *Boty* uses a self-priming mechanism with nine nucleotides (5′-TTTGAGCAC-3′) to initiate reverse transcript synthesis at Primer binding sites (PBSs) for first-strand reverse transcription. Based on the report of Lin and Levin(1997), we found that the *Boty* elements we identified used the same self-priming mechanism to initiate reverse transcript synthesis. The polypurine tract (PPT) corresponding to the PBS for plus-strand DNA synthesis was 5′-AGGCTAAGAAGGGGATAG-3′. *Boty* elements contained *gag–pol* genes encoding pol proteins with partial or complete AP, RT, RH, INT and chromatin organization modifier domain (CD) domains. In them, *boty-106* contains two identical LTRs and an internal *gag–pol* gene with *brtn*. The gene *gag–pol* encodes proteins with gag, ZnF_C2HC, RP, RT, IN, and CHROMO domains that is potentially functional (Fig. 1).

Using the putative gene *brtn* as a query, we detected 15 putative *brtn*-like genes of 492–618 bp, encoding putative BRTN proteins of 121–205 amino acids, in the *B. cinerea* B05.10 genome. All were flanked by a *Boty* LTR, so all *brtn*-like genes were located within *Boty*-like retrotransposons. Because of gaps in the genomic sequence and the availability of only partial *Boty* (GenBank Accession no: X81791) sequences with 1224 bp nucleotides, we could not determine if the other eight *Boty*-like retrotransposons and original *Boty* contained *brtn* genes. Since *Boty*-like retrotransposons without *brtn* were not identified, and all *brtn*-like genes were located within *Boty*-like retrotransposons, we propose that *brtn* is a common feature of *Boty*-like retrotransposons.

Expression analysis of brtn-like genes

Seven Expressed sequence tags (ESTs) from *B. cinerea* T4 with high similarity to *brtn*-like genes were detected (Supplementary material Table 2). This suggested that some *brtn*-like genes are potentially expressed. To verify this, we developed an RT-PCR analysis. Five *brtn* fragments (GenBank Accession no: FJ695493–FJ695497) were cloned from the mycelia of *B. cinerea* T4. We concluded that, at least in *B. cinerea* T4, some *brtn*-like genes are expressed.

Coding analysis of brtn

Four genomic DNA fragments with *brtn*-like genes and their 3′-LTRs were cloned from *B. cinerea* T4 genomic DNA using the primer pair brtn-s and LTR-a (GenBank Accession no: FJ695482, FJ695483, FJ695498, and FJ695499). Eight complete cDNAs of *brtn*-like genes were cloned using a RACE approach (GenBank Accession no: FJ695485–FJ695492). Because *Boty*-like elements are present as multiple copies in the genome, only one cDNA sequence (GenBank Accession no: FJ695486) and its parent genomic sequence (gene *brtn*1, GenBank Accession no: FJ695482) were cloned. The structural features and coding analysis of *brtn*1 are in Supplementary material Fig. 1. The *brtn*1 gene is 1380 bps, with an LTR of 480 bp (from 1 to 480), a gene with two exons coding for 173 amino acids (called BRTN1), and a 56-bp intron at the 3′-UTR. Gene *brtn*1 contains a PPT (5′-AGGCTAAGAAGGGGATAG-3′) adjacent to the LTR. Analysis of *brtn* revealed that it is transcribed in an antisense orientation to the *gag–pol* gene, and is located between *pol* and the 3′-LTR (Supplementary material Fig. 1).

The genomic locations and structural features of *Boty*-like elements, including the LTRs, GAG–POL proteins, PBS, PPT, target-site duplications, and gene *brtn* are in Supplementary Material Table 1. A schematic drawing of a complete *Boty*-like element (*Boty-106*) is in Fig. 1.

Predicted function prediction of BRTN

The predicted amino acid sequence of protein BRTN1 was used as the query in protein database similarity searches using BLASTP. Eleven hypothetical proteins in *B. cinerea* B05.10 and two deduced proteins in *S. sclerotiorum* 1980 showed significant similarities, sharing 56%–100% identity to BRTN1.

Multiple alignments of BRTN homologous sequences from different fungi revealed several conserved regions that may be important for protein function (Fig. 2). However, no known functional or structural protein domains were identified by RPS-BLAST, and no homology to any sequences of known function were identified by BLASTP. Thus, the function of BRTN remains unknown.

BLASTP searches showed that *BRTN* homologs exist in *S. sclerotiorum*. We performed data mining of *S. sclerotiorum* 1980 genomic sequences and identified a novel LTR retrotransposon in *S. sclerotiorum*. We named the identified sequence *ScscLRET* (*S. sclerotiorum* LTR retrotransposon). All BRTN homologs were detected in *ScscLRET*, and were located at positions similar to *brtn* (Supplementary material Table 3). This demonstrated that *ScscLRET* in *S. sclerotiorum* also contains an additional antisense *brtn*-like gene.

Retand-2 in *Silene latifolia*, *RIRE2* of rice, and *Grande1* of maize have ORFs of unknown function located at positions similar to *brtn* (Kumekawa et al., 1999; Neumann et al., 2003; Ohtsubo et al., 1999). However, we concluded that *Boty* is different from these plant elements because BRTN showed no similarities to these proteins, and in a neighbor-joining (NJ) tree based on RT-LTR domains, *Boty* formed a different clade than these elements (Supplementary material Fig. 2).

We identified *Boty* in *B. cinerea* and *ScscLRET* in *S. sclerotiorum* as containing additional antisense *brtn*-like genes. To assess the relationship of *Boty* and *ScscLRET*, we carried out phylogenetic analysis based on deuced amino acids of RT_LTR domains. Using a database search with "RT_LTR and fungi" as a query, and based on two reviews (Gorinšek et al., 2004; Kordis, 2005), we collected 151 complete fungal "RT_LTR" domains, containing 152–197 amino acids. The NJ phylogenetic tree is in Fig. 3a; with an enlargement of the *Boty* and *ScscLRET* clade in Fig. 3b. Phylogenetic analysis showed that the "RT-LTR" domains of *S. sclerotiorum* formed three clades, which suggested that *S. sclerotiorum*

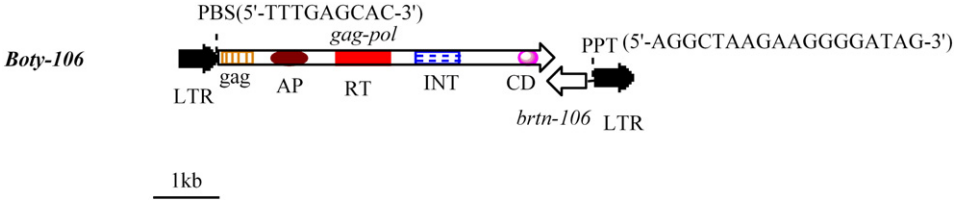

Fig. 1. Schematic drawing of *Boty-106* sequence elements. Non-coding region is ▬. Genes are ⇨. LTRs are indicated with ▶. Putative domains are gag, ▭; AP, ⬬; RT ▬; INT ▬; and CD ◯.

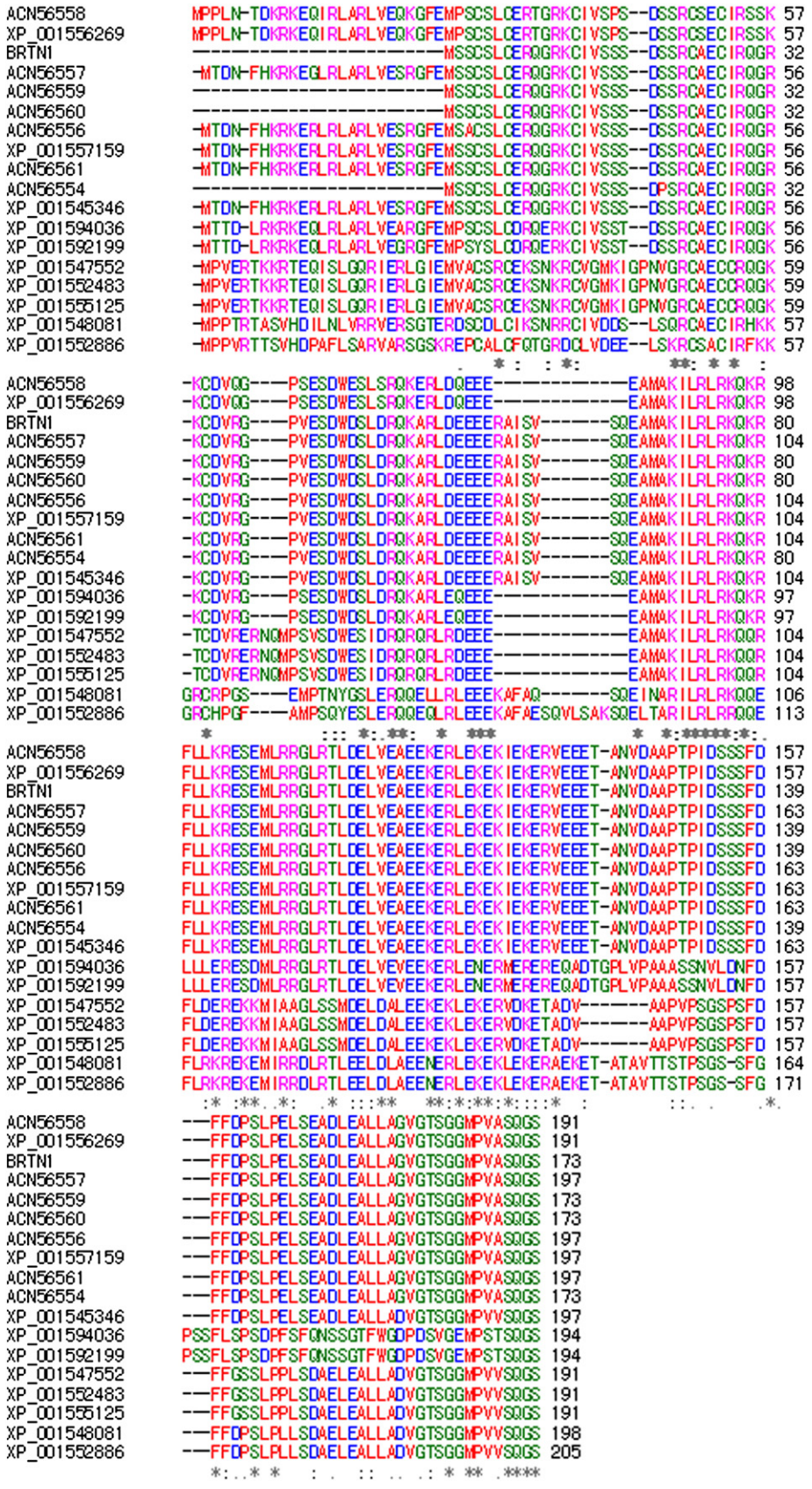

Fig. 2. Multiple alignments of BRTN homologs. *, residues are identical in all sequences in the alignment; conserved substitutions; semi-conserved substitutions.

might contain three different groups of LTR retrotransposons. One is *ScscLRET*; which formed a distinguished clade with *Boty* within the fungal LTR retrotransposons. *ScscLRET* contained a putative BRTN homolog, and from phylogenetic analysis, we concluded that *ScscLRET* elements belong to the *Boty* group. *B. cinerea* and *S. sclerotiorum* are necrotrophic plant pathogens that cause gray or white mold on

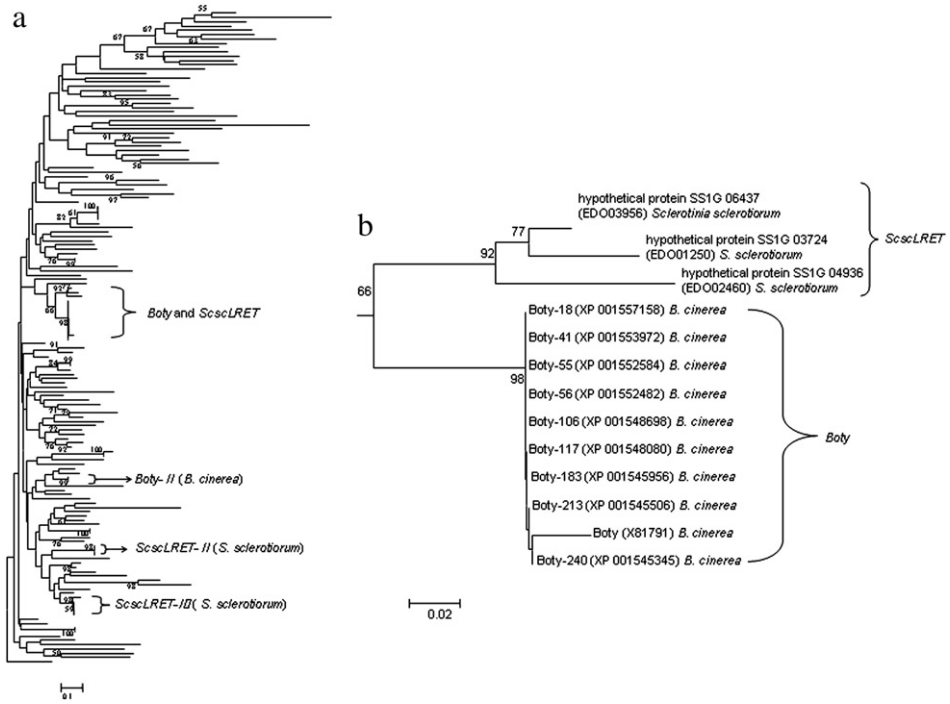

Fig. 3. Phylogenetic analysis of fungal LTR retrotransposons based on alignment of the RT_LTR domains. The tree was constructed by the NJ method (Saitou and Nei, 1987) and rooted using *Zea mays* LTR retrotransposons (ABQ44355) as the out-group. The NJ tree represents bootstrap consensus after 1000 replication. Nodes with confidence values greater than 50% are indicated. The deduced amino acid sequences of RT_LTR domains were from GenBank using RT_LTR and fungi as a query, or based on two reviews (Gorinšek et al., 2004; Kordis, 2005).

economically important crops (Billault et al., 2006). They belong to the family Sclerotiniaceae, and are very closely related (Holst-Jensen et al., 1997). Interestingly, in this work, we found that *B. cinerea* and *S. sclerotiorum* both contain *Boty*-like LTR retrotransposons; we hypothesize an interspecific horizontal transfer of *Boty* between *B. cinerea* and *S. sclerotiorum*.

We named the other LTR retrotransposons *ScscLRET-II* and *ScscLRET-III*. They formed different clades from each other, *ScscLRET*, *Boty*, and another LTR retrotransposon (*Boty-II*) in *B. cinerea* that was identified in a previous work (Fig. 3) (Zhao et al., 2009). Further survey of LTR retrotransposons in *S. sclerotiorum* and a comparison of LTR retrotransposons between *B. cinerea* and *S. sclerotiorum* are in progress. This comparison may offer an interesting opportunity to study the role of LTR retrotransposons in shaping the genomes of two closely related necrotrophic plant pathogens.

Conclusions

Through data mining of the *B. cinerea* genomic sequence and a RACE approach, we demonstrated that *Boty*-like retrotransposons in the filamentous fungus *B. cinerea* contain an additional antisense gene *brtn*, located between pol and the 3′-LTR, encoding a protein of unknown function. RT-PCR showed that, at least in *B. cinerea* T4, the gene *brtn* can be expressed. Homology searches found a novel *Boty*-like retrotransposons (*ScscLRET*) in *S. sclerotiorum*, which contain a *brtn*-like gene.

Materials and methods

Strains and nucleic acid extraction

B. cinerea T4 was kindly provided by Muriel Viaud (Viaud et al., 2005). Fungal genomic DNA was extracted as described by Möller et al. (1992). RNA was extracted using TriZOL reagent (Invitrogen, USA).

Survey of Boty-like elements in the B. cinerea B05.10 genome

The *Boty* LTR DNA sequence (GenBank Accession no: X81790) with internal *gag–pol* (GenBank Accession no: X81791) was used as a query to search the genome database of *B. cinerea* B05.10 by BLASTN search provided by the Broad Institute (http://www.broad.mit.edu/annotation/genome/botrytis_cinerea/Blast.html?sp=SBlastp) (Diolez et al., 1995). All BLAST hits with E value $<10^{-4}$ were extracted using Browse Region (http://www.broad.mit.edu/annotation/genome/botrytis_cinerea/Regions.html). Elements flanked by a pair of *Boty*-like LTRs and with an internal *gag*, *pol* or *gag–pol* gene were considered *Boty*-like retrotransposons and designated *Boty-xx*, where xx was the supercontig number in which the *Boty*-like element was detected. LTRs were designated *Boty*-xx L(R). Solo LTRs were named Sxx-solo.

PBSs and PPT were investigated based on the results of Diolez (Diolez et al., 1995), and Lin (Lin and Levin, 1997). The target-site duplications (TSD) of LTR retrotransposons were investigated by manual inspection. Diagrams of *Boty*-like elements were drawn with Chem Draw ultra 8.0.

Survey of brtn-like genes in the B. cinerea B05.10 genome

The DNA sequence of the putative gene *brtn* was used as a query to search the *B. cinerea* B05.10 genome database by BLASTN search, using the criteria and naming system described above. To investigate whether *brtn*-like genes are internal genes of *Boty*-like retrotransposons, the genomic locations of *brtn*-like genes and *Boty*-like LTRs were compared, and the annotated features of the up- and downstream flanking regions of *brtn*-like genes viewed using Browse Region (http://www.broad.mit.edu/annotation/genome/botrytis_cinerea/Regions.html).

Expression analysis of brtn-like genes

The expression of *brtn*-like genes was investigated by RT-PCR. First-strand cDNA was synthesized using EasyScript Reverse

Transcriptase (TransGen Biotech, China) with an oligo dT18 primer. The primers (Supplementary material table 4) used in RT-PCR were designed using ESTs sequences that showed high sequence similarity to the *brtn*-like genes detected by BLASTN searches provided by COGEME (http://cogeme.ex.ac.uk/blast.html) (Viaud et al., 2005).

Coding region analysis of brtn-like genes

Full-length DNA sequences of gene *brtn*-like genes were cloned using the primer pair *brtn*-s and LTR-a from T4 genomic DNA. The primers *brtn*-s and LTR-a were designed using the conserved motif of the INT domain and the right terminal of *Boty*-like elements (Supplementary material Table 2).The full-length cDNA sequences of *brtn*-like genes were cloned by 3′- and 5′-RACE using the Invitrogen RACE systems. The full-length cDNA and DNA sequences of *brtn*-like genes were compared using bl2seq to investigate the putative coding regions.

Function prediction of predicted protein BRTN

The deduced amino acid sequence of BRTN was used as a query to search the GenBank protein database by BLASTP search. Sequence hits with E value $<10^{-5}$, >50% identity and >50% coverage were considered homologs to the putative BRTN. The function of putative BRTN was predicted by BLASTP comparison with known function homologs, and by conserved protein domains search using RPS-BLAST.

Phylogenetic analysis

Phylogenetic analysis was performed on the basis of the deduced amino acid sequences of the RT_LTR domains (cd01647) of fungi LTR retrotransposons. The deduced RT domain amino acid sequences were obtained from GenBank using RT_LTR and fungi as a query, or based on two reviews (Gorinšek et al., 2004; Kordis, 2005). Multiple protein alignments and phylogenetic tree were constructed based on the alignment by MEGA version 5.05 (Tamura et al., 2007) using the NJ method (Saitou and Nei, 1987). The tree was subjected to bootstrap resampling of 1000 replicates. Default parameters were accepted for all programs.

Acknowledgments

This work was supported by the Key Project of Chinese Academy of Sciences (No. KSCX2-YW-G016 and No. KSCX2-EW-J-22) and the Hi-Tech Research and Development Program (863) of China (No. 2006AA10A213).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.virol.2011.06.020.

References

Billault, A., Choquer, M., Couloux, A., Cuomo, C., Decaprio, D., Dickman, M., Fillinger, S., Fournier, E., Galagan, J., Giraud, C., Kodira, C., Kohn, L., Levis, C., Mauceli, E., Pommier, C., Pradier, J.M., Quevillon, E., Rollins, J., Ségurens, B., Simon, A., Viaud, M., Weissenbach, J., Wincker, P., Lebrun, M.H., 2006. Whole genome sequencing of the fungal plant pathogen *Botrytis cinerea*: preliminary comparisons with Sclerotinia sclerotiorum genome. IXth International Fungal Biology Conference & 16th New Phytologist Symposium: Impact of genomics on fungal biology (18–20 September, 2006, Nancy, France), p. 24.

Bureau, T.E., White, S.E., Wessler, S.R., 1994. Transduction of a cellular gene by a plant retroelement. Cell 77, 479–480.

Diolez, A., Marches, F., Fortini, D., Brygoo, Y., 1995. *Boty*, a long-terminal-repeat retroelement in the phytopathogenic fungus *Botrytis cinerea*. Appl. Environ. Microbiol. 61, 103–108.

Gorinšek, B., Gubenšek, F., Kordiš, D., 2004. Evolutionary genomics of chromoviruses in eukaryotes. Mol. Biol. Evol. 21, 781–798.

Holst-Jensen, A., Kohn, L.M., Schumacher, T., 1997. Nuclear rDNA phylogeny of the Sclerotiniaceae. Mycologia 89, 885–889.

Jin, Y.K., Bennetzen, J.L., 1994. Integration and nonrandom mutation of a plasma membrane proton ATPase gene fragment within the Bs1 retroelement of maize. Plant Cell 6, 1177–1186.

Kejnovsky, E., Kubat, Z., Macas, J., Hobza, R., Mracek, J., Vyskot, B., 2006. Retand: a novel family of gypsy-like retrotransposons harboring an ampliwed tandem repeat. Mol. Genet. Genomics 276, 254–263.

Kim, A., Terzian, C., Santamaria, P., PéLisson, A., Prud-Homme, N., Bucheton, A., 1994. Retroviruses in invertebrates: the gypsy retrotransposon is apparently an infectious retrovirus of *Drosophila melanogaster*. Proc. Natl Acad. Sci. 91, 1285–1289.

Kordis, D., 2005. A genomic perspective on the chromodomain-containing retrotransposons: chromoviruses. Gene 347, 161–173.

Kumekawa, N., Ohtsubo, H., Horiuchi, T., Ohtsubo, E., 1999. Identification and characterization of novel retrotransposons of the gypsy type in rice. Mol. Gen. Genet. 260, 593–602.

Lin, J.H., Levin, H.L., 1997. Self-primed reverse transcription is a mechanism shared by several LTR-containing retrotransposons. RNA 3, 952–953.

Macas, J., Neumann, P., 2007. Ogre elements-A distinct group of plant Ty3/gypsy-like retrotransposons. Gene 390, 108–116.

Martíinez-Izquierdo, J.A., García-Martínez, J., Vicient, C.M., 1997. What makes Grande1 retrotransposon different? Genetica 100, 15–28.

Möller, E.M., Bahnweg, G., Sandermann, H., Geiger, H.H., 1992. A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi. Nucleic Acids Res. 20, 6115.

Neumann, P., Požárková, D., Macas, J., 2003. Highly abundant pea LTR retrotransposon Ogre is constitutively transcribed and partially spliced. Plant Mol. Biol. 3, 399–410.

Neumann, P., Koblížková, A., Navrátilová, A., Macas, J., 2006. Significant expansion of vicia pannonica genome size mediated amplification of a single type of giant retroelement. Genetics 173, 1047–1056.

Novikova, O.S., Blinov, A.G., 2008. dUTPase-containing metaviridae ltr retrotransposons from the genome of *phanerochaete chrysosporium* (fungi: Basidiomycota). Dokl. Biochem. Biophys. 420, 146–149.

Ohtsubo, H., Kumekawa, N., Ohtsubo, E., 1999. RIRE2, a novel gypsy-type retrotransposon from rice. Genes Genet. Syst. 74, 83–91.

Pérez-Alegre, M., Dubus, A., Fernández, E., 2005. REM1, a new type of long terminal repeat retrotransposon in *Chlamydomonas reinhardtii*. Mol. Cell. Biol. 25, 10628–10638.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4 (4), 406–425.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Viaud, M., Legeai, F., Pradier, J.M., Brygoo, Y., Bitton, F., Weissenbach, J., Brunet-Simon, A., Duclert, A., Fillinger, S., Fortini, D., Gioti, A., Giraud, C., Halary, S., Lebrun, I., Le-Pécheur, P., Samson, D., Levis, C., 2005. Expressed sequence tags from the phytopathogenic fungus *Botrytis cinerea*. Eur. J. Plant Pathol. 111, 139–146.

Vicient, C.M., Kalendar, R., Schulman, A.H., 2001. Envelope-class retrovirus-like elements are widespread, transcribed and spliced, and insertionally polymorphic in plants. Genome Res. 11, 2041–2049.

Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J.L., Capy, P., Chalhoub, B., Flavell, A., Leroy, P., Morgante, M., Panaud, O., Paux, E., San-Miguel, P., Schulman, A.H., 2007. A unified classification system for eukaryotic transposable elements. Nat. Rev. Genet. 8, 973–982.

Zhao, M., Zhou, J.Y., Li, Z.D., Song, W.W., Tan, Y.J., Tan, H., 2009. Boty-II, a novel LTR retrotransposon in *Botrytis cinerea* B05.10 revealed by genomic sequence. Electron. J. Biotechnol. 12 (3) fulltext-5.