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Amplification and cloning of putative reverse transcriptase genes from *Tricholoma* spp. by polymerase chain reaction

Hitoshi Murata[1)]* and Akiyoshi Yamada[2)]

[1)] Division of Bio-Resource Development, Forestry & Forest Products Research Institute, P.O.Box 16, Tsukuba-Norin, 305–8687, Japan

[2)] School of Agricultural Sciences, Shinshu University, Minami Minowa 8304, Nagano, 399–4598, Japan

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We previously identified a long terminal repeat retroelement from the ectomycorrhizal homobasidiomycete *Tricholoma matsutake*. In the present study, the reverse transcriptase gene, a hallmark of retroelements, was amplified by polymerase chain reaction from various species of ectomycorrhizal *Tricholoma*, and cloned. The results suggest that retroelements are shared in common in this fungal group rather than unique to *T. matsutake*.

Key Words——ectomycorrhizal basidiomycetes; retroelement; reverse transcriptase; *Tricholoma bakamatsutake*; *Tricholoma sejunctum*.

Retroelements are retrovirus-like DNA parasites acquired by eukaryotes in the course of their host evolution (Bingham and Zachar, 1989; Boeke, 1989; Hutchison III et al., 1989; Varmus and Brown, 1989). Retroelements incorporated into the host genome are inherited by the host progenies. In response to environmental stress, copies of retroelements are amplified and integrated into other genetic loci of their hosts through the activity of *pol* gene products, i.e., the reverse transcriptase (RT), RNase H (RN) and integrase (IN) (Fig. 1; Bingham and Zachar, 1989; Boeke, 1989; Varmus and Brown, 1989). We previously cloned an intact copy of the long terminal repeat (LTR) retroelement *marY1* from the ectomycorrhizal homobasidiomycete *Tricholoma matsutake* Y1 (Murata and Yamada, 2000). The objective of the present study is to explore the ubiquity of the retroelements in various species of ectomycorrhizal *Tricholoma*. The fungal strains used in this study are shown in Table 1. Nucleotide sequences described in this report have been deposited in DDBJ/GenBank/EMBL (Table 1).

To amplify a DNA segment encoding RT and RN from various species of *Tricholoma* by polymerase chain reaction (PCR), a pair of primers, 5′-GGACGTATTCGACCAT CA-3′ and 5′-ACGTCGGGATAGGGCATC-3′, was designed based respectively on the amino acid sequences GRIRPS in the N-terminal region of RT domain I and DALSRR in the C-terminal region of RN of *marY1* (Fig. 1; Murata and Yamada, 2000). PCR was conducted by using the primers, templates extracted from species of *Tricholoma* and a high fidelity Taq reaction mixture (LA Taq, Takara Shuzo Co., Ohtsu, Japan) as described (Murata et al., 1999). With the annealing temperature of 50°C, PCR products were generated in samples of *Tricholoma magnivelare* Tp-C3 and *Tricholoma sejunctum* NA12 which correspond to the 1.1-kb *rt-rn* fragment of the *marY1* (Fig. 1; Murata and Yamada, 2000). In the sample of *Tricholoma bakamatsutake* B1, a unique 2.0-kb fragment was amplified, in which the 5′-primer associated with a sequence 0.9 kb further upstream of the expected sequence, while the 3′-primer annealed as expected (data not shown).

These fragments were ligated into the TA cloning vector pCR2.1 (Invitrogen, Carlsbad, CA, USA), transformed into *Escherichia coli* strain JM109, and extracted from four randomly selected Ap^r, $LacZ^-$ colonies per sample. Nucleotide sequences of cloned fragments were determined with an ABI prism 377 autosequencer (Applied Biosystems, Foster City, CA, USA) and analyzed by use of the computer software GENETIX-Mac ver. 9.0 (Software Development Co, Tokyo, Japan), Clustal X (Thompson et al., 1997) and Advanced Blast Search provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). All fragments cloned from *T. magnivelare* Tp-C3, *T. sejunctum* NA12 and *T. bakamatsutake* B1 are predicted to encode an amino acid sequence that is 91%, 80% and 55% identical to that of *marY1*, respectively (Fig. 2).

A second pair of primers, 5′-AATCGATACCCGCT GCCGCTCATT-3′ and 5′-CCAGTGAGCCTGTCTGCAAT TTAG-3′, was designed based respectively on the amino acid sequences NRYPLPLI in the N-terminal region of RT domain II and LNCRQAHW in the C-terminal region of RN of *T. sejunctum* NA12 (Fig. 1). These amino acid sequences are highly conserved between *marY1* of *T. matsutake* Y1 and a putative retroelement of *T. sejun-*

* Corresponding author: Division of Mushroom Sciences & Applied Microbiology, Forestry & Forest Products Research Institute, P.O. Box 16, Tsukuba-Norin, 305–8687, Japan.
TEL; +81(0)298–73–3211. E-mail; murmur@ffpri.affrc.go.jp.

Table 1. List of the fungal strains and nucleotide sequences described in this report.

Organisms	DEBJ/GenBank/EMBL accession no.	Type of retroelement
Tricholoma matsutake Y1 (IFO 33136)*	AB028236	M1 element (*marY1*)
Tricholoma matsutake Y1 (IFO 33136)*	AB039862	M2 element
Tricholoma bakamatsutake B1 (IFO 33138)*	AB036886	M2 element
Tricholoma portentosum 615 (IFO 33144)*	AB036887	M1 element
Tricholoma fulvocastaneum MR26	AB036889	M1 element
Tricholoma magnivelare Tp-C3	AB036885	M1 element
Tricholoma magnivelare Tp-C3	AB039863	M2 element
Tricholoma sejunctum NA12	AB036888	M1 element

* These fungal strains have been deposited in the culture collection of the Institute for Fermentation, Osaka, and the IFO accession number is shown. For the description of fungal strains, see Murata et al. (1999).

ctum NA12. With an annealing temperature of 50°C, 1.0-kb products were generated in samples of *Tricholoma fulvocastaneum* MR26 and *Tricholoma portentosum* 615 that correspond to the fragment of *T. sejunctum* NA12, *T. matsutake* Y1 and *T. magnivelare* Tp-C3. Nucleotide sequencing was conducted in four randomly selected clones of each PCR product resulting from ligation with the vector pCR2.1. The putative amino acid sequences encoded in *T. fulvocastaneum* MR26 and *T. portentosum* 615 have 63% and 67% identity to *marY1*, respectively (Fig. 2). Like *marY1*, all these amino acid sequences carry YLDDILF in the putative active domain V, and therefore retroelements correspond to this class were designated as M1 elements (Figs. 1, 2).

A third pair of primers, 5′-AATGCGCACCCTTTGC CATTGATC-3′ and 5′-CCAACGTGCTTGTCGATGGTTA AG-3′, was designed based respectively on the amino acid sequences NAHPLPLI and LNHRQARW encoded in the fragment cloned from *T. bakamatsutake* B1, rather than the corresponding sequences NRYPLPL and LNCRQAHW in *T. sejunctum* NA12 (Fig. 1). PCR with these primers (annealing temperature = 50°C) generated the 1.0-kb fragment only in samples of *T. bakamatsutake* B1, *T. matsutake* Y1 and *T. magnivelare* Tp-C3. Nucleotide sequencing revealed that the putative amino acid sequences of *T. matsutake* Y1 and *T. magnivelare* Tp-C3 were 92–93% identical to that of *T. bakamatsutake* B1, and 56–57% identical to *marY1* (Fig. 2). This phenomenon is relevant to the notion that there are at least two types of retroelements in *T. matsutake*. Unlike M1 elements, putative retroelements shared by *T. matsutake, T. magnivelare* and *T. bakamatsutake* carry the amino acid sequence FIDDILF in a putative active domain V, and therefore retroelements belonging to this class were designated as M2 elements (Figs. 1, 2).

The ubiquity of retroelements was demonstrated in some species of *Tricholoma*. According to the current concept of retroelement evolution, retrotransposons are horizontally transferred across major taxonomic groups of organisms, subsequently evolved into new types of elements by interacting with pre-existing retroelements, and vertically transmitted to the lineage (Flavell and Smith, 1992; Flavell et al, 1992; Kumar, 1996; Mizrokhi and Mazo, 1990; Voytas et al., 1992; Xiong and Eickbush, 1990). It is generally accepted that the degree of divergence in the *rt* gene, a hallmark of retroelements, correlates with the evolutionary distance among host organisms (Flavell and Smith, 1992; Flavell et al, 1992; Kumar, 1996; Mizrokhi and Mazo, 1990; Voytas et al., 1992; Xiong and Eickbush, 1990). In addition, active

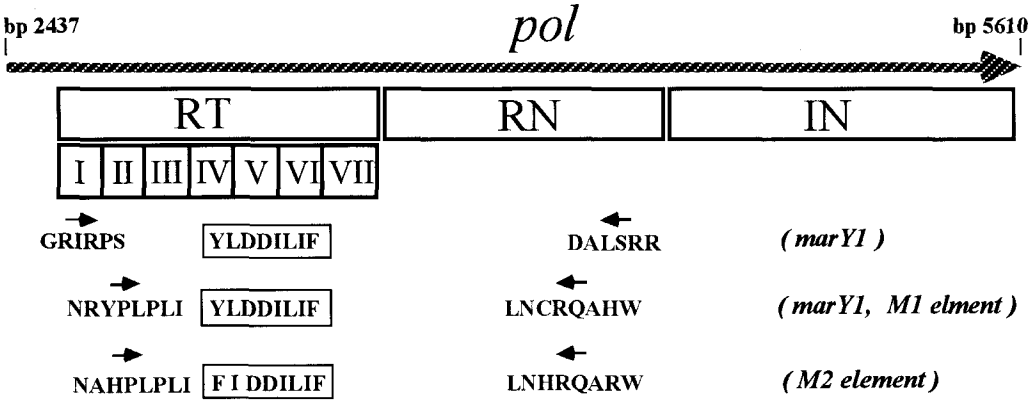

Fig. 1. Schematic representation of *pol*, encoding reverse transcriptase (RT), RNase H (RN) and integrase (IN), and location of amino acid sequences used for the preparation of primers. The hatched bold arrow depicts the *pol* gene. RT domains I–VII (I–VII) are given. Solid arrows aligned with the representation of the RT and RN domains indicate the direction and the location of amino acid sequences used to design primers for PCR-cloning. Amino acid sequences are shown by the single-letter code, and those conserved in the putative active domain V are boxed in. Nucleotide sequence position (bp) relative to the 5′ end of *marY1* is given.

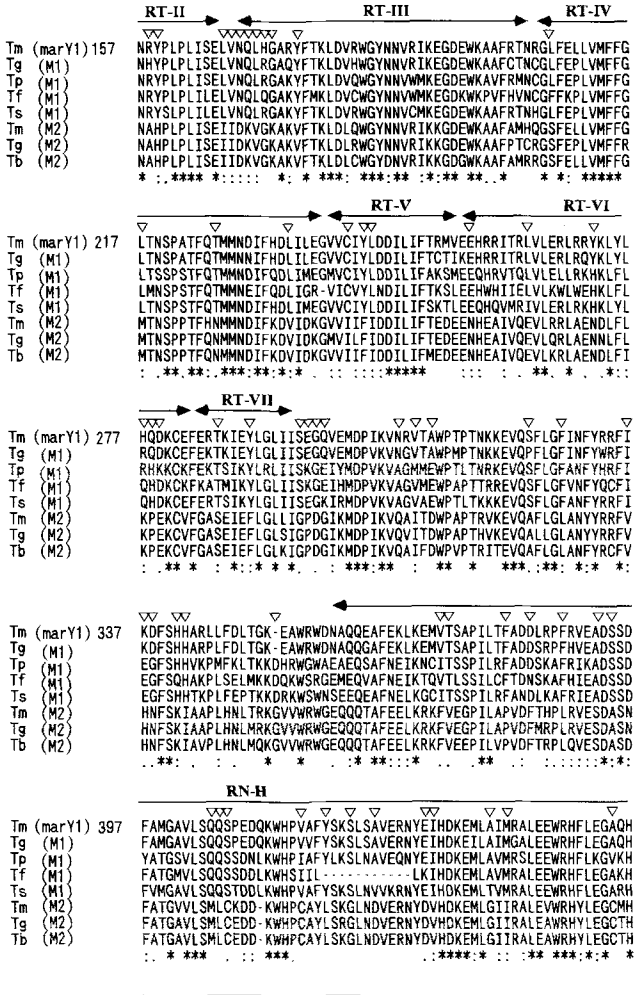

Fig. 2. Amino acid alignment (single-letter amino acid code) of functional domains characteristic of putative retroelements cloned from ectomycorrhizal *Tricholoma*. Alignment was conducted by using the CLUSTAL X program (Thompson et al., 1997): *=positions which have a single fully conserved residue, :=one of the "strong" groups fully conserved, .=one of the "weaker" groups fully conserved. Reverse transcriptase domains II–VII (RT-II, RT-III, RT-IV, RT-V, RT-VI and RT-VII), RNase H (RN-H) and the amino acid position of the *marY1 pol* gene product are indicated. Amino acid residues that could differentiate the M2 element from *marY1* or the M1 element are marked with an open triangle. Abbreviations: Tm=*T. matsutake* Y1, Tg=*T. magnivelare* Tp-C3, Tf=*T. fulvocastaneum* MR26, Tp=*T. portentosum* 615, Ts=*T. sejunctum* NA12, Tb=*T. bakamatsutake* B1, M1=M1 element, M2=M2 element.

retroelements are regarded as mutators that generate variations among host species (Bingham and Zachar, 1989; Finnegan, 1989; Mizrokhi and Mazo, 1990; Mizrokhi et al., 1985; Nishimura et al., 2000). Therefore, retroelements are useful not only as markers to analyze molecular evolution of the host organisms but also as tools to generate mutants and recombinants (Flavell and Smith, 1992; Flavell et al., 1992; Kumar, 1996; Lucas et al., 1995; Nishimura et al., 2000; Xiong and Eickbush, 1990). It is still not certain how many kinds of retroelements are present in the fungi belonging to *Tricholoma*, which retroelements are active as transposons, and to what extent "DNA-parasites" were involved in the evolution of plant-associating fungi. Advances in retroelement-based investigation, however, will provide further insights into the molecular evolution of ectomycorrhizal fungi and new approaches to investigate molecular mechanisms involved in the symbiosis.

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