Botanical Studies (2007) 48: 377-385.

Characterization of MRT, a new non-LTR retrotransposon in *Monascus* spp.

Yi-Pei CHEN[1,2], Ching-Ping TSENG[2], Li-Ling LIAW[1], Chun-Lin WANG[1], and Gwo-Fang YUAN[1,*]

[1]*Bioresource Collection and Research Center, Food Industry Research and Development Institute, P.O. Box 246, HsinChu 300, Taiwan*

[2]*Department of Biological Science and Technology, National Chiao Tung University, HsinChu, Taiwan*

(Received October 11, 2006; Accepted June 25, 2007)

ABSTRACT. A new non-LTR retrotransposon, named MRT, was discovered in the filamentous fungus *Monascus pilosus* BCRC38072. The entire nucleotide sequence of the MRT element was 5.5-kb long, including two open reading frames. These two ORFs showed homologies to *gag*-like and *pol*-like gene products, and an A-rich sequence at the 3′ end of *pol*-like gene. ORF1 encoded a protein of 517 amino acids and contained a cysteine-rich zinc finger motif. ORF2 encoded a protein of 1181 amino acids and contained apurinic/apyrimidinic endonuclease (APE), reverse transcriptase (RT), RNaseH domains, and a CCHC motif. The phylogenetic analyses demonstrated that the MRT element should be classified into the Tad1 clade. The results of Southern hybridizations showed that MRT elements were distributed within *M. pilosus*, *M. ruber*, *M. sanguineus*, and *M. barkeri*. In addition, the species of *Monascus* can be grouped by the presence or absence of MRT elements in the hybridization pattern according to phylogenetic subgroups established with the partial β-tubulin gene.

Keywords: Bacterial artificial chromosome; *Monascus pilosus*; Non-LTR retrotransposon; Phylogenetic analysis.

INTRODUCTION

Retrotransposons are found in most eukaryotes and in some cases constitute a major part of the genome (e.g. 40-50% of the human genome). They have been divided into two subclasses based on their differences in overall structure. LTR retrotransposons closely relate to retroviruses and non-LTR retrotransposons, also called LINE-like elements. All these elements use reverse transcription to propagate. Non-LTR retrotransposons have been found in many groups of eukaryotic organisms, including mammals, insects, amphibians, plants, and also fungi.

Five non-LTR retrotransposons have been characterized in filamentous fungi, including *Tad1-1* in *Neurospora crassa* (Cambareri et al., 1994), MGR583 in *Magnaporthe grisea* (Hamer et al., 1989), CgT1 in *Colletotrichum gloeosporioides* (He et al., 1996), *marY2N* in *Tricholoma matsutake* (Murata et al., 2001), and Mars1 in *Ascobolus immerses* (Goyon et al., 1996) though the entire element is not described for this last species. These non-LTR retrotransposons usually contain two ORFs encoding *gag*-like and *pol*-like proteins. Moreover, they generally have poly (A) or A-rich regions at their 3′ terminus and generate truncation in 5′ UTRs. Phylogenetic analysis of non-LTR retrotransposons based on the RT (reverse transcriptase) domain, the only sequence found in all elements, defined eleven clades by Malik et al. (1999). More recently, Burke et al. (2002) proposed an additional classification in which the various clades fall into five groups on the basis of both the phylogenetic relationship of their RT sequence and the nature and arrangement of their protein domains. Based on sequence, structure, and phylogenetic analyses, the non-LTRs elements from filamentous fungi are grouped in the Tad1 clade. Recently, two non-LTR retrotransposons of yeast, Zorro in *Candida albicans* (Goodwin et al., 2001) and Ylli in *Yarrowia lipolytica* (Casaregola et al., 2002), have been placed into the L1 clade of mammalian elements.

According to the distribution of CgT1 in *C. gloeosporioides*, the presence or absence of CgT1 can be used to distinguish biotypes A and B that cause different anthracnose diseases on *Stylosanthes* in Australia (He et al., 1996). Moreover, DNA fingerprint analysis of CgT1 reveals that Australian isolates of biotype B are monomorphic. In addition, the analysis of the genetic relations and evolutionary history of many species has been facilitated by repetitive DNA fingerprinting probe (Cizeron et al., 1998; Blesa et al., 2001; Daboussi and Capy, 2003).

*Corresponding author: E-mail: gfy@firdi.org.tw; Tel: +886-3-5223191 ext. 580; Fax: +886-3-5224171.

Monascus spp. belongs to the ascomycetes, and has been used in Chinese fermented foods such as anka, anka pork, and rice wine for thousands of years. Thirteen *Monascus* species have been reported. They are known as producers of various secondary metabolites with polyketide structures, such as monacolins, and have medical importance (Endo, 1979). In this study, we report the discovery of a new non-LTR retrotransposon named MRT (*Monascus* Retrotransposon) in *Monascus* spp. The structural, genomic and phylogenetic analysis of the MRT elements was presented. Moreover, the distribution of MRT in *Monascus* species was also analyzed.

MATERIALS AND METHODS

Strains, media, and growth conditions

The nineteen strains of *Monascus* listed in Table 1 were used in this study. All strains were maintained on YM (DIFCO, Detroit, Michigan) agar for one week, and spore suspensions were obtained by washing cultured YM agar plates with distilled water. Mycelia for DNA isolation were harvested from YM broth after incubating for 8 days at 28°C with constant agitation and then frozen at -80°C.

BAC library construction and shotgun sequencing

The methods of Peterson et al. (2000) were used to make a BAC library of *Monascus pilosus* BCRC 38072. DNAs of eleven BAC clones were extracted for shotgun sequencing by a Qiagen Large-Construct kit (Qiagen, Valencia, CA). DNA sequencing was performed with an ABI Prism 3700 Sequencer (Applied Biosystems, Foster City, CA). The Phred-Phrap-Consed system developed by the Phil Green Laboratory was used to assemble DNA fragments (Gordon et al., 2001). Nucleotide and deduced amino acid sequences were used to interrogate the non-redundant database at GenBank using BlastN and BlastX. Sequence analysis was done using VectorNTI 9.0 (InforMax, Frederick, MD) software. Prediction of nucleic acid secondary structure was performed with Mfold server (http://bioweb.pasteur.fr/seqanal/interfaces/mfold-simple.html). The nucleotide sequences of MRT non-LTR retrotransposons found in this study have been submitted to GenBank under the accession numbers AY900582 and DQ299897 to DQ299900.

Genomic DNA preparation and Southern hybridization

Monascus genomic DNA was extracted according to the method developed by Bingle et al. (1999). Approximately 0.5 g (squeezed wet weight) of frozen mycelia was ground to a fine power under liquid nitrogen using a mortar and pestle. Protein was removed by successive rounds of extraction with phenol and chloroform. Genomic DNA

Table 1. Strains used and GenBank accession numbers for the β-tubulin gene.

Strain	Species[a]	MRT non-LTR retrotransposon[b]	Accession number of β-tubulin gene
BCRC 38072 (Taiwan isolate)	*Monascus pilosus*	+	DQ299886
BCRC 31502 (ATCC 16363)	*Monascus pilosus*, Type	+	AY498596
BCRC 31503 (ATCC 16364)	*Monascus pilosus*	–	DQ299887
BCRC 31533 (ATCC 16246)	*Monascus ruber*, Type	+	AY498589
BCRC 31523 (ATCC 16378)	*Monascus ruber*	+	DQ299888
BCRC 31534 (ATCC 16366)	*Monascus ruber*	+	AY498587
BCRC 31535 (ATCC 18199)	*Monascus ruber*	+	DQ299889
BCRC 33314 (ATCC 16371)	*Monascus ruber*	+	AY498588
BCRC 33323 (ATCC 18199)	*Monascus ruber*	+	DQ299890
BCRC 31542 (ATCC 16365)	*Monascus purpureus*, Type	–	DQ299891
BCRC 31541 (ATCC 16379)	*Monascus purpureus*	–	AY498598
BCRC 31615 (DSM 1379)	*Monascus purpureus*	–	DQ299892
BCRC 33325 (IFO 30873)	*Monascus purpureus*	–	DQ299893
BCRC 31506 (CBS 302.78)	*Monascus kaoliang*, Type	–	DQ299894
BCRC 33446 (ATCC 200613)	*Monascus sanguineus*, Type	+	AY498602
BCRC 33309 (ATCC 16966)	*Monascus barkeri*	+	DQ299895
BCRC 33310 (IMI 282587)	*Monascus floridanus*, Type	–	DQ299896
BCRC 33640 (ATCC 204397)	*Monascus lunisporas*, Type	–	AY498604
BCRC 33641 (ATCC 200612)	*Monascus pallens*, Type	–	AY498601

[a]"Type" indicates type strain.
[b]+, presence of MRT non-LTR retrotransposon; –, absence of MRT non-LTR retrotransposon.

was recovered by precipitation with ethanol and dissolved in TE buffer. For Southern hybridizations, genomic DNA (7.5 µg per lane) was digested with *Eco*RI and *Bam*HI restriction enzymes and separated through 1.0% agarose gels by electrophoresis. Southern hybridization analysis was performed using the DIG system (Roche Diagnostics, Mannheim, Germany). The probe of the MRT element was labeled by PCR amplification from genomic DNA of *M. pilosus* 38072 using a PCR DIG probe synthesis kit (Roche Diagnostics, Mannheim, Germany). The primer set of the MRT probe was MRT1-1F: CAGGGGGAGGCTAGGATGTA, and MRT1-1R: CACAGGTGGGTAGAGCCACAG. All other DNA manipulations were performed as described in Sambrook et al. (1989).

Phylogenetic analysis

In addition to MRT element, β-tubulin gene was chosen for phylogenetic analysis of *Monascus* spp. The partial β-tubulin genes were amplified with the primer set, btubulinF: 5′-CAACTGGGCTAAGGGTCATT and btubulinR: 5′-GTGAACTCCATCTCGTCCATA (Wu et al., 1996; Park et al., 2004). Sequences of partial β-tubulin genes obtained from *Monascus* strains used in this study have been submitted to GenBank under the accession numbers DQ299886 to DQ299896. The other accession numbers of partial β-tubulin genes, AY498587 to AY498589, AY498596, AY498598, AY498601, AY498602 and AY498604, were obtained from the GenBank database. The phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using MEGA 3.1 software with 1000 bootstrap replicates.

RESULTS AND DISCUSSION

During the whole genome sequencing of *M. pilosus* BCRC38072, two repetitive sequences (mps01-1 and mps01-2) were observed in a ca. 160 kb BAC, mps01. They were found to have a homology similar to CgT1 (He et al., 1996), a non-LTR retrotransposon from *Colletotrichum gloeosporioides* (mps01-1, 31% identity and mps01-2, 32% identity by BlastX). The sequence homology indicated that the repetitive sequences were non-LTR retrotransposons. The new non-LTR retrotransposon was designated MRT. Eleven BAC clones of *M. pilosus* BCRC38072 were sequenced covering 1.55 Mb; and six positive BACs were identified, and 15 copies of the MRT element were found in determining the relative abundance and diversity of the non-LTR retrotransposon. The entire nucleotide sequence of the MRT element was 5.5-kb long. Given that the size of the *M. pilosus* BCRC38072 genome was ~30 Mb, the number of copies of the genome was estimated to be ~290, which occupied about 5% of the *M. pilosus* BCRC38072 genome.

The translation frames of the entire set of MRT elements revealed that four of them in BACs—mps02-1, mps07-1, mps11-1, and mps13-1—contained two

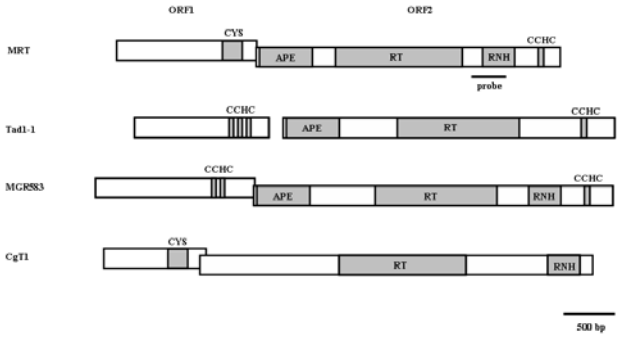

Figure 1. Structure of the MRT element. Schematic representation of the structural organization of the filamentous fungi non-LTR retrotransposons. Tad1-1, MGR583 and CgT1 in *Neurospora crassa*, *Magnaporthe grisea* and *Colletotrichum gloeosporioides* were obtained from the GenBank database using the following accession numbers, L25662, AF018033 and L76205, respectively. Southern hybridization analysis of MRT in the genomes of *Monascus* species hybridized with the probe indicated by small black bar. The abbreviation of CYS indicated the cysteine-rich region, RNH indicated the RNaseH, APE indicated the apurinic/apyrimidinic endonuclease domain, RT indicated the reverse transcriptase domain, and CCHC indicated the Cys-His region.

open reading frames (Figure 1), like other non-LTR retrotransposons that have been found in fungi, CgT1, Tad-1, and MGR583 (Hamer et al., 1989; Cambareri et al., 1994; He et al., 1996). Numerous stop codons were found in other copies of the MRT elements. A conceptual translation demonstrated that the first ORF in the MRT element may encode a protein of 517 amino acids. The deduced amino acid sequence of the MRT ORF1 contained one zinc finger motif that was cysteine-rich (Figures 1 and 2). The arrangement of cysteines indicated that the consensus sequence $CX_6HXCX_6CHX_2HX_6C$ represented an NF-X1-type zinc finger, based on Pfam analysis (Song et al., 1994). However, no 5′ UTR was clearly identified, suggesting that all of the active element may be rare in *M. pilosus* BCRC38072. A conceptual translation demonstrated that the second ORF in the MRT element may encode a protein of 1181 amino acids. The ORF1 and ORF2 overlapped by 1 bp. The deduced amino acid sequence of the MRT ORF2 contained an apurinic/apyrimidinic endonuclease (APE) domain, a reverse transcriptase (RT) domain, an RNaseH domain, and a CCHC motif (Figures 1 and 3). The APE domain at the N-terminal of ORF2 in the MRT element was proven not to be well conserved in other organisms. The RT domain located downstream of the APE domain contained conservation of the deduced amino acid sequences, YXDD, which were a part of the active site in the RT domain (He et al., 1996). The 3′ UTRs of the MRT elements were around 300 bp, and the 3′ ends were well conserved. Two parts, the A-rich sequence and the stem-loop region, were present in the conserved tail (Figure 4A). Interestingly, the A-rich stretch represented two sequences,

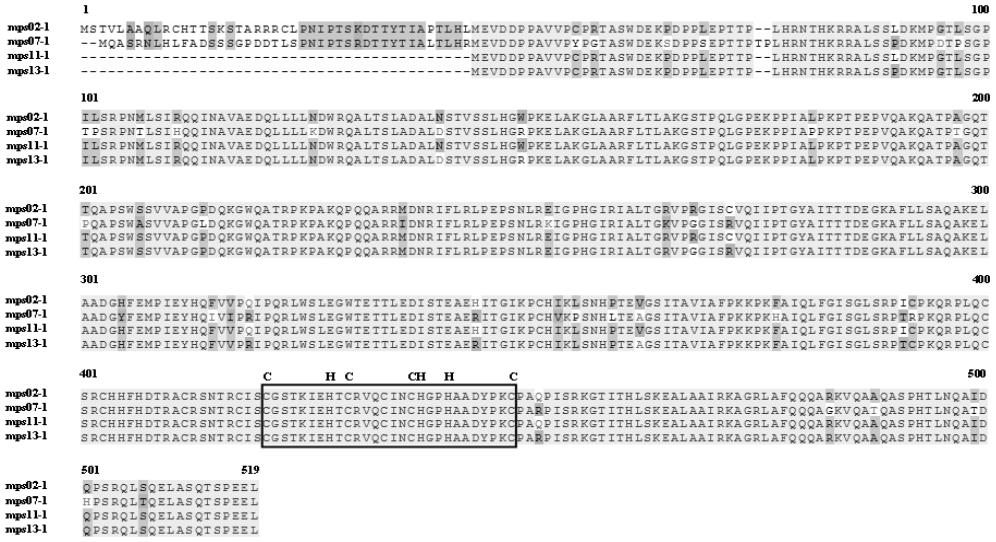

Figure 2. Deduced Amino acid sequences alignment of the MRT elements ORF1. The cysteine-rich nucleotide-binding domain represented a zinc finger with the consensus sequence $CX_6HXCX_6CHX_2HX_6C$ shown boxed.

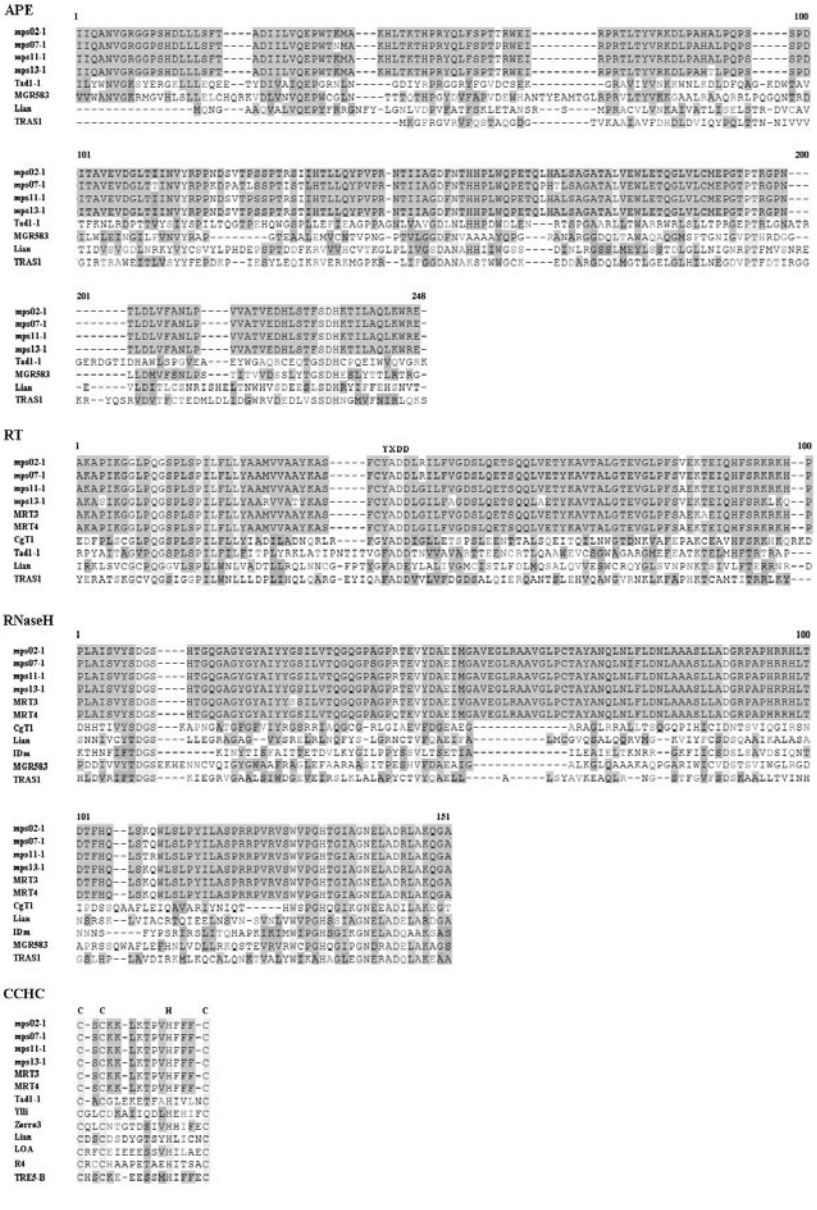

Figure 3. Multiple alignment of deduced amino acid sequences of the MRT elements with related organisms. Comparison of the N-termial apurinic/ apyrimidinic endonuclease (APE) domains based on an alignment of approximately 210 amino acid residues. Comparison of the reverse transcriptase (RT) domain. Conserved YXDD residues, active site of reverse transcriptase domain, were indicated above the alignment. Comparison of the RNaseH domains based on an alignment of approximately 130 amino acid residues. Conserved residues of RNaseH were described by Malik et al. (1999). Comparison of the C-terminal Cys-His region (CCHC). Conserved $CX_1CX_7HX_3C$ residues, putative zinc finger of the MRT element, were indicated above the alignment.

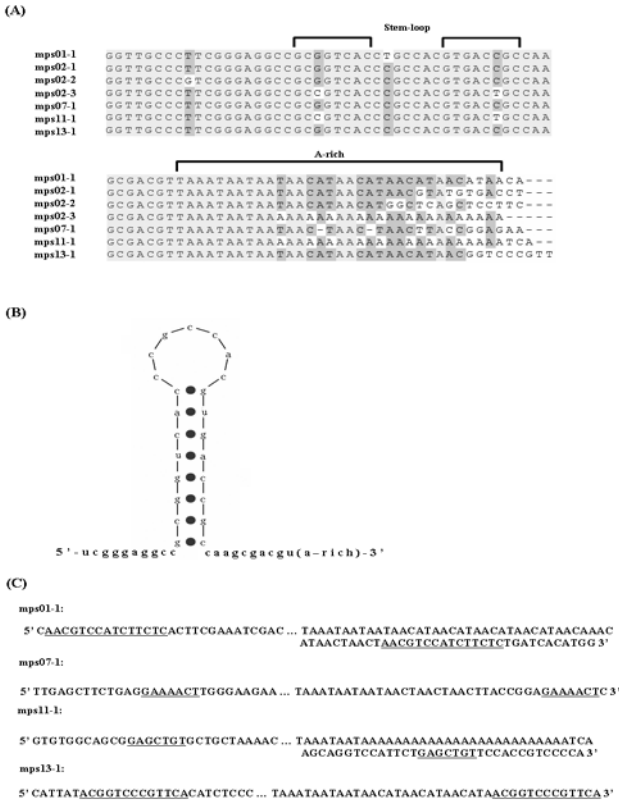

Figure 4. (A) Comparison of 3′ conserved region of the MRT elements from BACs. The A-rich sequences and stem-loop were marked. (B) Putative secondary structure of 3′ conserved region of the MRT element RNA as indicated in (A) was depicted. (C) Sequences at the ends of the MRT elements. Four elements from BACs were shown. Putative target site duplications were indicated by underlining.

TAAATAATAA(CATAA)n and TAAATAATAA(A)n. Additionally, the RNA transcribed from the conserved tail of the MRT element was proposed to form a stem-loop (Figure 4B). This stem-loop region can be recognized by the reverse transcriptase of the non-LTR retrotransposon (Baba et al., 2004). Furthermore, most non-LTR retrotransposons have target site duplications (TSDs) of variable lengths from 4 to 49 bp (Eickbush, 1992). The sequence results also revealed that four MRT elements presented 7 to 15 bp target site duplications (Figure 4C).

Since reverse transcriptase (RT) is a fundamental component of the machinery required to synthesize DNA, it is strongly conserved and used in analyses of retrotransposon phylogeny (Flavell, 1995). In particular, the eleven conserved block sequences of the reverse transcriptase domain defined by Malik et al. (1999) are extensively used for the construction of retrotransposon phylogeny. This study analyzed the phylogenetic relationships between members of the non-LTR retrotransposons, including the four MRT elements described above, using shared reverse transcriptase domains. The phylogenetic tree was rooted using RT sequences of bacterial and fungal group II introns (Xiong and Eickbush, 1990). Eleven clades were clearly distinguished (Figure 5A). This result was consistent with the phylogeny constructed by Malik et al. (1999). The phylogenetic tree further suggested that the MRT element belonged to the Tad1 clade known from filamentous fungi. Moreover, the apurinic/apyrimidinic endonuclease (APE) domain is believed to cleave DNA in the reverse transcription reaction at the chromosome target site (Maita et al., 2004) and is commonly employed to construct the phylogenetic analysis (Malik et al., 1999). Since CRE,

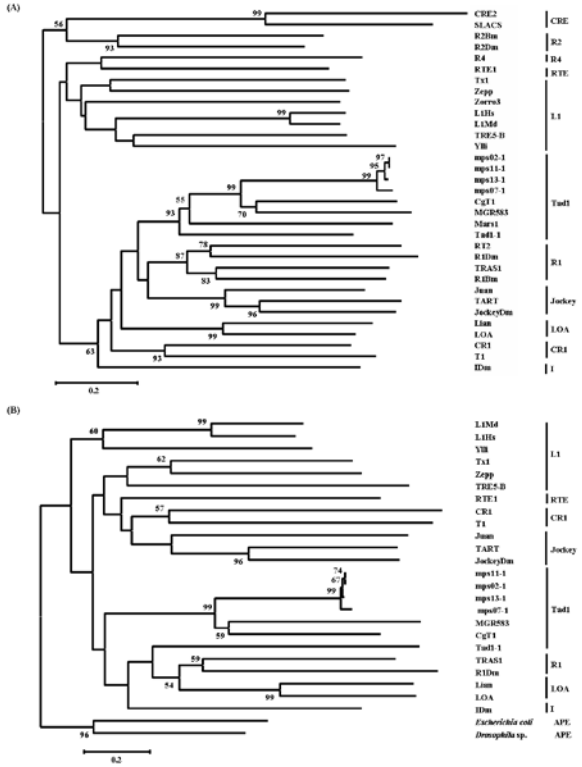

Figure 5. Phylogenetic tree of non-LTR retrotransposons from *M. pilosus* and various organisms. (A) The phylogeny of non-LTR retrotransposons based on the eleven conserved blocks of the reverse transcriptase domains defined by Malik et al. (1999) was constructed. The nucleotide sequences of MRT non-LTR retrotransposons were used in this study under the accession numbers DQ299897 to DQ299900. The tree was rooted using RT sequences of bacterial and fungal group II introns with *Lactococcus lactis* (P0A3U0), *Escherichia coli* (NP053121), *Sinorhizobium meliloti* (CAC49872) *Neurospora crassa* (NP041729), *Saccharomyces cerevisiae* (NP009310), and *Schizosaccharomyces pombe* (S78199). (B) The phylogeny of non-LTR retrotransposons based on an alignment of approximately 210 amino acid residues of apurinic/apyrimidinic endonuclease (APE) domain. Accession numbers for the apurinic/apyrimidinic endonuclease used as the outgroup following: *Drosophila* sp. (AAB19427), and *Escherichia coli* (P09030). Bootstrap values were shown in the nodes according to the 1000 replications. Only bootstrap values >50 were shown. The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987).

R2 and R4 clades lacked an APE domain, other clades were applied to construct a phylogenetic tree that was rooted using the apurinic/apyrimidinic endonuclease of *Drosophila* sp. (AAB19427), and *Escherichia coli* (P09030). Figure 5B depicts the phylogenetic tree of the non-LTR retrotransposons according to the APE domain. It demonstrates that the close relationship between members of the Tad1 clade is consistent with the phylogeny obtained from the reverse transcriptase domain. The APE phylogeny had lower resolution than the RT phylogeny, perhaps because that APE domain was smaller and less conserved than the RT domain (Malik et al., 1999). Additionally, even though the APE and RT domains of CgT1 contained termination codons in the ORF1 and ORF2 (He et al., 1996), the phylogeny analysis of the deduced amino acid sequence of the CgT1 element also revealed that belonged to the Tad1 clade.

In order to study the distribution of MRT elements in *Monascus* species (Table 1), their genomic DNA was extracted and digested by *Eco*RI and *Bam*HI. The results of Southern hybridization showed that the MRT elements were widely distributed over *M. pilosus*, *M. ruber* and *M. barkeri* (Figure 6), and a large number of high-intensity bands were detected in these species. In contrast, the MRT element was absent in the species of *M. purpureus*, *M. kaoliang*, *M. floridanus*, *M. lunisporas*, and *M. pallens*. The intensity and diversity of hybridization patterns also shown in *M. sanguineus*, a newly found species of *Monascus*, were weaker than those in the species of *M. pilosus*, *M. ruber*, and *M. barkeri*. The weaker intensity of bands implied that the copy number of MRT in *M. sanguineus* was lower than in *M. ruber*, *M. pilosus*, and *M. barkeri*. The fingerprints of the DNA hybridizations demonstrated that the band patterns were distributed between 500 bp to 10 kb. However, the species *M. pilosus* BCRC31503 was an exception that could not detect the presence of any MRT elements (Figure 6). A phylogenetic characterization using the partial β-tubulin gene as a molecular differentiation marker was conducted to elucidate the evolutionary history of MRT elements among different species. According to the study of Park et al. (2004), the partial β-tubulin gene can be adopted to examine the phylogenetic relationship among the *Monascus* species without gaps in the alignment of partial β-tubulin genes. *Aspergillus flavus* (M38265), *Aspergillus parasiticus* (L49386), *Aspergillus fumigatus* (AY048754) and *Penicillium digitatum* (D78154) were used as outgroups of phylogenetic analysis. Interestingly, the result of the phylogenetic analysis of the partial β-tubulin gene showed that *M. pilosus* BCRC31503, *M. purpureus*, and *M. kaoliang* were placed into the same clade (Figure 7). This finding was in agreement with the results obtained by grouping species into a Southern hybridization pattern by the presence or absence of MRT elements. Therefore, *M. pilosus* BCRC31503 may have been misidentified and should be reconsidered as *M. purpureus*. Since *M. pilosus*, *M. ruber*, and *M. barkeri* could not be differentiated using the partial β-tubulin genes, three species have been suggested to be synonymous, meaning that they should be classified as a single species. DNA hybridization among

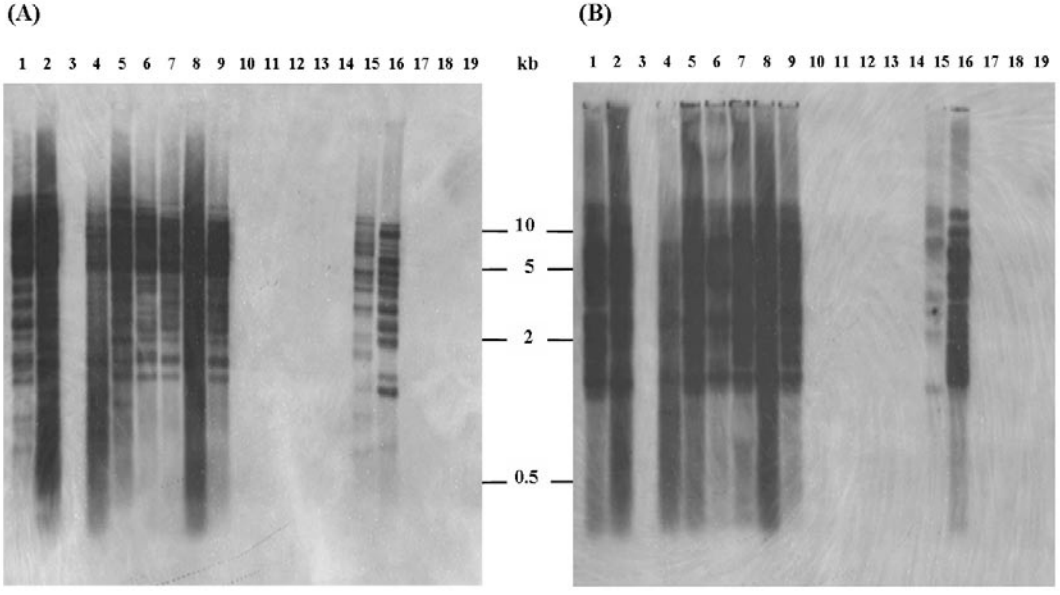

Figure 6. Southern hybridizations analyses of the MRT elements. Chromosome DNAs extracted from *Monascus* species were digested by *Eco*RI (A) and *Bam*HI (B) separated on electrophoresis gel and hybridized respectively with 422-bp probe. *Monascus* species—lane 1: *M. pilosus* BCRC38072; lane 2: *M. pilosus* BCRC31502; lane 3: *M. pilosus* BCRC31503; lane 4: *M. ruber* BCRC31533; lane 5: *M. ruber* BCRC31523; lane 6: *M. ruber* BCRC31534; lane7: *M. ruber* BCRC31535; lane 8: *M. ruber* BCRC33314; lane 9: *M. ruber* BCRC33323; lane 10: *M. purpureus* BCRC31542; lane 11: *M. purpureus* BCRC31541; lane 12: *M. purpureus* BCRC31615; lane 13: *M. purpureus* BCRC33325; lane 14: *M. kaoliang* BCRC31506; lane 15: *M. sanguineus* BCRC33446; lane 16: *M. barkeri* BCRC33309; lane 17: *M. floridanus* BCRC33310; lane 18: *M. lunisporas* BCRC33640; lane 19: *M. pallens* BCRC33641.

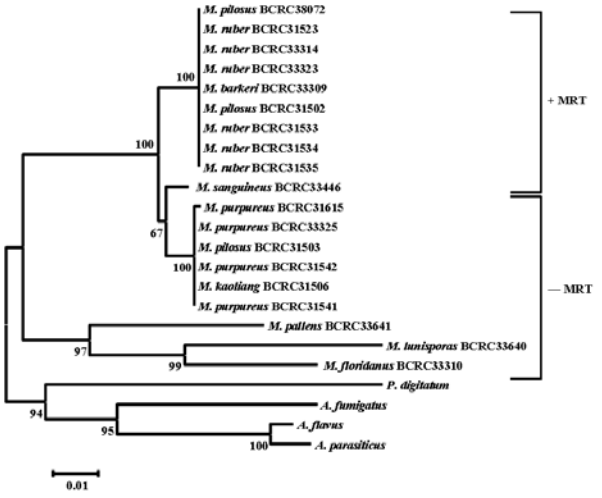

Figure 7. Phylogeny of *Monascus* species based on the partial β-tubulin gene amplified by PCR. The partial β-tubulin genes were used by the following accession numbers, DQ299886 to DQ299896, AY498587 to AY498589, AY498596, AY498598, AY498601, AY498602 and AY498604. Accession numbers for the β-tubulin genes were used as the outgroup following: *Aspergillus flavus* (M38265), *Aspergillus parasiticus* (L49386), *Aspergillus fumigatus* (AY048754), and *Penicillium digitatum* (D78154). Bootstrap values were shown in the nodes according to the 1000 replications. Only bootstrap values >50 were shown. The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987). + MRT: presence of MRT non-LTR retrotransposon; – MRT: absence of MRT non-LTR retrotransposon.

Monascus species (our unpublished data) also supported the identity of *M. pilosus* and *M. ruber*. The other genetic markers have been found to distinguish *Monascus* species using the D1/D2 region of the large subunit (LSU) rRNA genes (Park and Jong, 2003) and the ITS region (Park et al., 2004). These results also demonstrate that *M. ruber* and *M. pilosus* could not be differentiated. Moreover, the MRT non-LTR retrotransposons were widely distributed over *M. pilosus* and *M. ruber*. Hence, the two species were determined to be synonymous. In the taxonomy of *Monascus* (Hawksworth and Pitt, 1983), *M. barkeri* goes by the name of *M. ruber.* The results herein were consistent with their theory. Furthermore, *M. sanguineus* was placed on a branch that was separate from *M. pilosus*, *M. ruber*, and *M. barkeri,* while the difference of phylogenetic distance corresponded to the results of Southern hybridization with varying intensities of the bands of *M. pilosus*, *M. ruber*, and *M. barkeri*.

According to the phylogenetic subgroups established with the partial β-tubulin gene, the species were grouped by the presence or absence of MRT elements in the hybridization pattern (Figure 7). Since the MRT element was only detected in *M. pilosus*, *M. ruber*, *M. barkeri* and *M. sanguineus*, the MRT element was suggested to have been present in the ancestors of the *Monascus* species, and absent from most other species or to have diverged from *M. pilosus*. This phenomenon may have been caused by the genetic drift that is itself associated with small effective populations, which are responsible for an increase in the numbers of copies of elements in some species and a decline in others (Cizeron et al., 1998; Le Rouzic and Capy, 2005). However, horizontal transfer (HT) has been observed with the variable distribution of retrotransposons between different classes, phyla, or kingdoms. The horizontal transfer is believed to be present in members of the RTE clade (Župunski et al., 2001). Bov-B LINEs of the RTE clade exhibit a very low divergence between Ruminantia and Squamata, strongly indicating horizontal transfer. In this study, the result with the various species of *Monascus* revealed that MRT non-LTR retrotransposon was restricted to *M. sanguineus*, *M. pilosus*, *M. ruber*, and *M. barkeri*, the last three species of which were synonymous. These observations suggested that the MRT element was introduced into the ancestor of the *Monascus* species, and then diverged into *M. sanguineus*, *M. pilosus*, *M. ruber*, and *M. barkeri*. Although the evidence for horizontal transfer events is present in non-LTR retrotransposons of RTE clade, different taxonomic groups must be sampled to determine whether the horizontal transfer events also occurred in the Tad1 clade.

Transposable elements may be regarded as genetic components that evolve in an ecological community of the host genome (Brookfield, 1995). During evolution, the spread and distribution of the elements depend on not only their ability to amplify, but also the complex interactions between various families of elements, and between the elements and the hosts (Tu et al., 1998). Analyses of endogenous retrotransposable elements in *Monascus* species can be potentially used as markers in genetic mapping and in population studies. MRT was consistent with CgT1, which can distinguish between two groups based on presence or absence of non-LTR retrotransposon (He et al., 1996). Practically, our findings indicate that MRT has great potential in strain characterization and in studies of population structure and evolution in *Monascus* spp.

Acknowledgments. Support of the Ministry of Economic Affairs, Taiwan, ROC, (Grant No. 94-EC-17-A-17-R7-0563) to FIRDI is appreciated.

LITERATURE CITED

Baba, S., M. Kajikawa, N. Okada, and G. Kawai. 2004. Solution structure of an RNA stem-loop derived from the 3′ conserved region of eel LINE UnaL2. RNA **10:** 1380-1387.

Bingle, L.E.H., T.J. Simpson, and C.M. Lazarus. 1999. Ketosynthase domain probes identify two subclasses of fungal polyketide synthase genes. Fungal Genet. Biol. **26:** 209-223.

Blesa, D., M. Gandía, and M.J. Martínez-Sebastián. 2001. Distribution of the *bilbo* non-LTR retrotransposon in Drosophilidae and its evolution in the *Drosophila obscura*

species group. Mol. Biol. Evol. **18:** 585-592.

Brookfield, J.F. 1995. Molecular evolution. Retroposon revivals. Curr. Biol. **5:** 255-256.

Burke, W.D., H.S. Malik, S.M. Rich, and T.H. Eickbush. 2002. Ancient lineages of non-LTR retrotransposons in the primitive eukaryote, *Giardia lamblia*. Mol. Biol. Evol. **19:** 619-630.

Cambareri, E.B., J. Helber, and J.A. Kinsey. 1994. *Tad1-1*, an active LINE-like element of *Neurospora crassa*. Mol. Gen. Genet. **242:** 658-665.

Casaregola, S., C. Neuvéglise, E. Bon, and C. Gaillardin. 2002. Ylli, a non-LTR retrotransposon L1 family in the dimorphic yeast *Yarrowia lipolytica*. Mol. Biol. Evol. **19:** 664-677.

Cizeron, G., F. Lemeunier, C. Loevenbruck, A. Brehm, and C. Biémont. 1998. Distribution of the retrotransposable element 412 in *Drosophila* species. Mol. Biol. Evol. **15:** 1589-1599.

Daboussi, M.J. and P. Capy. 2003. Transposable elements in filamentous fungi. Annu. Rev. Microbiol. **57:** 275-299.

Eickbush, T.H. 1992. Transposing without ends: the non-LTR retrotransposable elements. New Biol. **4:** 430-440.

Endo, A. 1979. Monacolin K, a new hypocholesterolemic agent produced by a *Monascus* species. J. Antibiot. (Tokyo) **32:** 852-854.

Flavell, A.J. 1995. Retroelements, reverse transcriptase and evolution. Comp. Biochem. Physiol. **110:** 3-15.

Goodwin, T.J., J.E. Ormandy, and R.T. Poulter. 2001. L1-like non-LTR retrotransposons in the yeast *Candida albicans*. Curr. Genet. **39:** 83-91.

Gordon, D., C., Desmarais, and P. Green. 2001. Automated finishing with autofinish. Genome Res. **4:** 614-625.

Goyon, C., J.L. Rossignol, and G. Faugeron. 1996. Native DNA repeats and methylation in *Ascobolus*. Nucleic. Acids. Res. **24:** 3348-3356.

Hamer, J.E., L. Farrall, M.J. Orbach, B. Valent, and F.G. Chumley. 1989. Host species-specific conservation of a family of repeated DNA sequences in the genome of a fungal plant pathogen. Proc. Natl. Acad. Sci. USA **86:** 9981-9985.

Hawksworth, D.L. and J.I. Pitt. 1983. A new taxonomy for *Monascus* species based on cultural and microscopical characters. Aust. J. Bot. **31:** 51-65.

He, C., J.P. Nourse, S. Kelemu, J.A. Irwin, and J.M. Manners. 1996. CgT1: a non-LTR retrotransposon with restricted distribution in the fungal phytopathogen *Colletotrichum gloeosporioides*. Mol. Gen. Genet. **252:** 320-331.

Le Rouzic, A. and P. Capy. 2005. The first steps of transposable elements invasion: parasitic strategy vs. genetic drift. Genetics **169:** 1033-1043.

Maita, N., T. Anzai, H. Aoyagi, H. Mizuno, and H. Fujiwara. 2004. Crystal structure of the endonuclease domain encoded by the telomere-specific long interspersed nuclear element, TRAS1. J. Biol. Chem. **279:** 41067-41076.

Malik, H.S., W.D. Burke, and T.H. Eickbush. 1999. The age and evolution of non-LTR retrotransposable elements. Mol. Biol. Evol. **16:** 793-805.

Murata, H., Y. Miyazaki, and A. Yamada. 2001. *marY2N*, a LINE-like non-long terminal repeat (non-LTR) retroelement from the ectomycorrhizal homobasidiomycete *Tricholoma matsutake*. Biosci. Biotechnol. Biochem. **65:** 2301-2305.

Park, H.G., E.K. Stamenova, and S-C Jong. 2004. Phylogenetic relationships of *Monascus* species inferred from the ITS and the partial β-tubulin gene. Bot. Bull. Acad. Sin. **45:** 325-330.

Park, H.G. and S.-C. Jong. 2003. Molecular characterization of *Monascus* strains based on the D1/D2 regions of LSU rRNA genes. Mycoscience **44:** 25-32.

Peterson, D.G., J.P. Tomkins, D.A. Frisch, R.A. Wing, and A.H. Paterson. 2000. Construction of plant bacterial artificial chromosome (BAC) libraries: An illustrated guide. J. Agric. Genomics. Volume 5.

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

Sambrook, J., E. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor, Cold Spring Harbor Laboratory Press, New York.

Song, Z., S. Krishna, D. Thanos, J.L. Strominger, and S.J. Ono. 1994. A novel cysteine-rich sequence-specific DNA-binding protein interacts with the conserved X-box motif of the human major histocompatibility complex class II genes via a repeated Cys-His domain and functions as a transcriptional repressor. J. Exp. Med. **180:** 1763-1774.

Tu, Z., J. Isoe, and J.A. Guzova. 1998. Structural, genomic, and phylogenetic analysis of Lian, a novel family of non-LTR retrotransposons in the yellow fever mosquito. *Aedes aegypti*. Mol. Biol. Evol. **15:** 837-853.

Wu, T.S., C.D. Skory, J.S. Horng, and J.E. Linz. 1996. Cloning and functional analysis of a beta-tubulin gene from a benomyl resistant mutant of *Aspergillus parasiticus*. Gene **182:** 7-12.

Xiong, Y. and T.H. Eickbush. 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J. **9:** 3353-3362.

Županski, V., F. Gubenšek, and D. Kordiš. 2001. Evolutionary dynamics and evolutionary history in the RTE clade of non-LTR retrotransposons. Mol. Biol. Evol. **18:** 1849-1863.

MRT，紅麴菌中新穎的 non-LTR 逆轉位子之特徵分析

陳煜沛[1,2]　曾慶平[2]　廖麗玲[1]　王俊霖[1]　袁國芳[1]

[1]食品工業發展研究所

[2]國立交通大學生物科技系所

本研究於紅麴菌中發現新的 non-LTR 逆轉位子，並命名為 MRT。其序列長度約為 5.5 kb，包含有兩個開放讀架 (ORFs)。此兩個開放讀架與 gag 及 *pol* 基因相似，並且於 *pol* 相似基因的 3′ 端具有很多的腺嘌呤。第一個開放讀架為 517 個氨基酸的蛋白質，包含有多量半胱氨酸的鋅指區域。第二個開放讀架為 1181 個氨基酸的蛋白質，包含有脫嘌呤/脫嘧啶核酸內切酶、反轉錄酶、核糖核酸酶 H 及 CCHC 區域。根據 MRT non-LTR 逆轉位子的氨基酸序列所建立的親緣關係，可將其歸屬於 Tad1 群叢。南方雜交法則進一步發現只有四種紅麴菌 *M. pilosus*，*M. ruber*，*M. sanguineus* 與 *M. barkeri* 具有 MRT non-LTR 逆轉位子。除此之外，利用 β-tubulin 基因所建立的親緣關係則可歸群出 MRT non-LTR 逆轉位子之存在與否。

關鍵詞：紅麴菌；細菌人工染色體基因庫；Non-LTR 逆轉位子；親緣分析。