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A Group I Intron in the 18S Ribosomal DNA from the Parasitic Fungus *Isaria japonica*

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Abstract. The nucleotide sequence of the 18S rDNA coding gene in the ascomycetes parasitic fungus *Isaria japonica* contains a group I intron with a length of 379 nucleotides. The identification of the DNA sequence as a group I intron is based on its sequence homology to other fungal group I introns. Its group I intron contained the highly conserved sequence elements P, Q, R, and S found in other group I introns. Surprisingly, the intron sequence of *I. japonica* is more similar to that of *Ustilago maydis* than to the one found in *Sclerotinia sclerotiorum.* This is in contrast to the sequence identity found on the neighboring rDNA. This is an interesting finding and suggests a horizontal transfer of group I intron sequences.

Key words: 18S rDNA — Group I intron — *Isaria japonica* — Fungal evolution

Introduction

Cordyceps species, which are parasitic fungi on insects, belong to the order Hypocreales in Ascomycetes. *Cordyceps* species infect larva or imago of insects, kill them, and then form a fruit body on the insect. The host selectivity is strict. Some *Cordyceps* species, including *Cordyceps sinensis*, are used as Chinese traditional medicines in Japan and China. Further, it has been reported that *Cordyceps* species produce bioactive compounds with antibacterial, antifungal, and immunosuppressive activity (Kneifel et al. 1977; Furuya et al. 1983; Fujita et al. 1994). The classification of *Cordyceps* species have been proposed by Shimizu (1994) based mainly on morphological properties, color, shape, and host insect, but their phylogeny has not been established. Recently, in the classification field, the analytical methods have drastically changed from morphological to molecular genetic techniques. Among bacteria and eukaryotes, the comparison of ribosomal RNA (rDNA) sequences is the most useful method for deducing the phylogenetic relationships (Woese 1987; Gutell 1993; Wilmotte et al. 1993).

Method

For *Cordyceps* species this method has not been applied and their phylogenetic relationship with other fungi is not known. For the purpose of analyzing the DNA sequence, it is necessary to amplify it by polymerase chain reaction (PCR) method because it can amplify any particular DNA sequence region by use of a pair of primers (Boettger 1989; Medlin et al. 1988; Edwards et al. 1989). We designed new primers and analyzed the 18S rDNA of three *Cordyceps* species, *Cordyceps tuberculata, Isaria japonica,* and *Hymenostilbe odonatae* (Ito and Hirano 1996). Isaria species and Hymenostilbe species are anamorphs of Cordyceps species. The PCR product of the 18S rRNA gene from *I. japonica* only was found to be larger (~2.1 kb) than expected from its coding region (~1.8 kb), so we suspected that a insertion was contained in the 18S rDNA of *I. japonica.* In order to determine the location of the proposed insertion, we designed five primers based on the nucleotide sequence of the 18S rDNA from *Sclerotinia sclerotirum,* which belongs to Ascomycetes, the division containing *Cordyceps* species. By these primers we identified the position of the insertion in 18S rDNA of *I. japonica.* Also the nucleotide sequence of the insertion was determined. The insertion sequence was shown to be a group I intron by DNA homology search. Introns can

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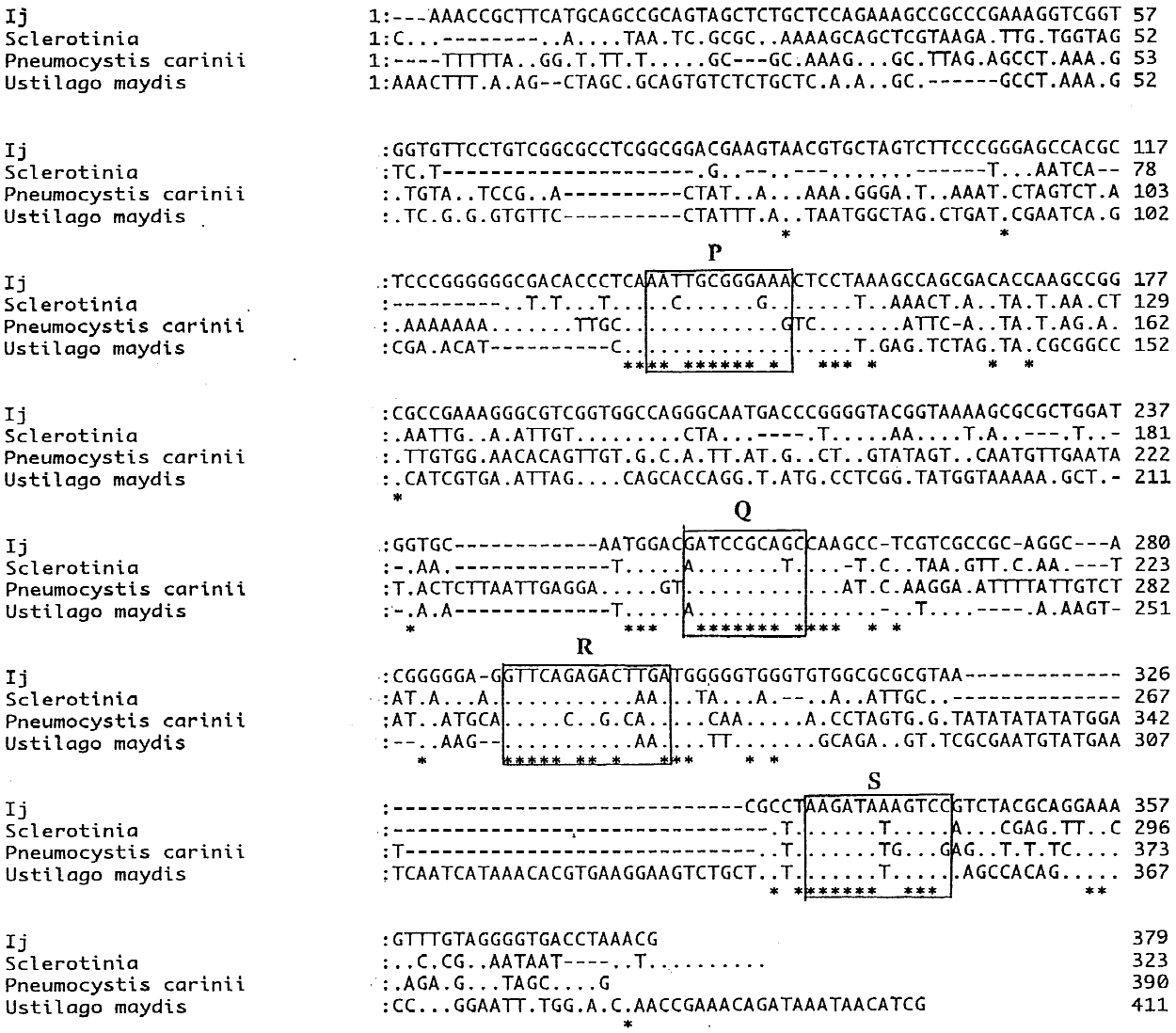

Fig. 1. Alignment of group I introns in 18S rDNA. The catalytic core regions (P, Q, R, S) are indicated *above* the sequences. The identical nucleotides are shown by *dots*, and alignment gaps by *dashes.* The conserved nucleotides are indicated by *asterisks.* Ij, *Isaria japonica;* Sclerotinia, *Sclerotinia sclerotiorum.*

catalyze their own excision from the rRNA precursor and rejoin the two exons to produce the mature rRNA. Group I introns are characterized by conserved RNA secondary structures essential for splicing and are often capable of self-splicing or require protein factors for excision (Cech 1988). Group I introns have often been found in the nuclear and organellar genomes of fungi, green algae, and higher plants (Bhattacharya et al. 1994). The origin and distribution of group I introns are not understood. *Protomyces inouyei, Pneumocystis carinii, Ustilago maydis,* and *S. sclerotiorum* have been reported to contain I introns in their 18S rDNA (Nishida et al. 1993; Sogin et al. 1989; De Wachter et al. 1992; Wilmotte et al. 1993). So we compared the nucleotide sequence of the *I. japonica* group I intron with that of other fungi, and assessed the distribution of group I introns in 18S rRNA gene among higher fungi.

Results and Discussion

Isaria japonica Yasuda, *Hymenostilbe odonatae* Y. Kobayasi, and *Cordyceps tuberculata* (Leb.) Maire f. moelleri (Henn.) Y. Kobayasi were obtained from insects captured in Ibaraki Prefecture in Japan. *Cordyceps sinensis* (Berk.) Sacc. was purchased as dried material at a Chinese drug store in Tokyo. *I. japonica* is an anamorph of *Cordyceps takaomontana* Yakushiji et Kumazawa. The distribution is restricted mainly to Asia. *Isaria felina* ATCC 26680 and *Isaria sulfrea* ATCC 22280 were obtained from ATCC (American Type Culture Collection) and cultivated in Sabouraud dextrose agar medium (Difco Laboratories, Detroit, MI). We extracted DNAs from *C. sinensis, I. japonica, H. odonatae, C. tuberculata, I. felina,* and *I. sulfurea* (Jhingan 1992) and amplified them with several kinds of PCR primer sets. The nucleotide numbers of PCR primers are equivalent to those in the primary structure of *S. sclerotiorum* MUCL11553 (Wilmotte et al. 1993). By primers F-2 (5′CGACTTCGGAAGGGGTGTATTTATT3′, corre-

Table 1. Sequence homologies among the four fungi[a]

Strain	% homology 1	2	3	4
1. *I. japonica*	100			
	100			
2. *S. sclerotiorum*	59.2	100		
	91.5	100		
3. *P. carinii*	50.3	55.0	100	
	86.1	86.8	100	
4. *U. maydis*	62.3	66.3	53.9	100
	79.7	82.6	80.3	100

[a] *I, Isaria; S, Sclerotinia; P, Pneumocystis; U, Ustilago.* The upper-right triangle indicates introns; and the lower-left, 18S rDNA.

sponding to bp positions 183–207 of the 18S rRNA gene from *Sclerotinia sclerotiorum*) and R-1 (5′TAATGATCCTTCCGCAGGTT3′, corresponding to 2107–2088), almost the entire 18S rDNA could be amplified, and we found that the size of the PCR product from *I. japonica* was larger than that of the product from the other fungi. The primer set F-7 (5′TGACAGATTGAGAGCTCTTTCTTGA3′; corresponding to 1538–1562) and R-1 could amplify the downstream region including phylogenetically changeable region in 18S rDNA. We showed all PCR products to be identical in size. By the primer F-6 (5′GACGATCAGATACCGTCGTAGTC3′, corresponding to 992–1014) and R-2 (5′GGTCTCGTTCGTTATCGCAATTAAG3′, corresponding to 1639–1615), we could amplify the central region in the 18S rDNA. The PCR product from *I. japonica* was larger than the other products. Thus screening by means of PCR amplification detected the intron in the 18S rDNA of *I. japonica*, but not in the other members of the *Cordyceps* species.

We determined the nucleotide sequence of the insertion in 18S rDNA from *I. japonica* by using the primers F-6 and R-2. The sequencing reaction was carried out by the cycle sequencing method described by Pharmacia LKB Biotechnology (Uppsala, Sweden). The length of the insertion sequence was 379 nucleotides (Fig. 1). Representative sequences of the strains used in this study were deposited in the DNA Data Bank of Japan (DDBJ) as D86057. By homology search of DNA data banks, this insertion was shown to be a group I intron. This insertion sequence was compared with that in the 18S rDNA of nuclear genome from three other fungi. The alignment of group I introns from four fungi, i.e., *I. japonica, S. sclerotiorum* (Wilmotte et al. 1993), *P. carinii*, which causes pneumonia, and *U. maydis*, are shown in Fig. 1. The inserted sequences were aligned by use of the GENETYX version 8 program (Software Development Co., LTD., Tokyo). The 18S rRNA sequences and the inserted sequences were obtained from the EMBL database, X62396 for *Ustilago maydis*, X12708 for *Pneumocystis carinii*, and X69850 for *Sclerotinia sclerotiorum. I. japonica* is an anamorph of *Cordyceps takaomontana* and belongs to the Hypocreales in Ascomycetes. *P. carinii* belongs to the Pneumocystidales in Ascomycetes; *S. sclerotiorum*, to the Leotiales in Ascomycetes; and *U. maydis*, to the Ustilaginales in Basidiomycetes. The sequence of P, Q, R, and S regions are highly conserved sequence elements in group I introns (Cech 1988), and the group I intron of *I. japonica* was shown to have the same conserved regions (Fig. 1). These regions were also highly conserved among the other three fungi as compared with other sequence regions. Also, the group I intron in the 18S rDNA from *I. japonica* was found at the same position (nt 943, relative to the *Escherichia coli* SSU rRNA coding region) as the intron in the gene from *S. sclerotiorum* and *U. maydis.*

Recently four common insertion sites (nt 943, 1046, 1506, 1512) in 18S rRNA gene have been proposed for group I introns in fungi, green algae, and red algae (Bhattacharya et al. 1994). Introns of fungi have been found in the ‘‘nt 943’’ and/or ‘‘nt 1506’’ position. We have supposed the positions to be structurally appropriate for insertion of the introns.

The homology of group I intron sequences among four fungi are shown in the upper-right triangle in Table 1. Interestingly, the group I intron of *I. japonica* was found to be more similar to the intron of the basidiomycetous fungus *U. maydis* (62.3%) than to the introns of the two other ascomycetous fungi (*S. sclerotiorum*, 59.2%; *P. carinii*, 50.3%). Also, the intron sequence of *U. maydis* was similar to that of the ascomycetous fungus *S. sclerotiorum* (66.3%). The intron sequence of the ascomycetous fungus *P. carinii* showed a low similarity to the other three fungi. Further, the nucleotide sequences of partial 18S rDNA from the four fungi were compared (the lower-left triangle in Table 1). The compared sequence region contained about 500 bp of the upstream from the 3′ end of 18S rDNA and included the variable regions V7-9 corresponding to the nucleotide sequences of *S. cerevisiae.* It has been reported that the nucleotide sequences in variable regions differ among fungi and that the sequences reflect phylogenetic relationship (Neefs et al. 1993). The sequence of *I. japonica* showed a higher similarity to that of *S. sclerotinia* (91.5%) than to that of *U. maydis* (79.7%). Thus *I. japonica* was phylogenetically close to the ascomycete *S. sclerotinia* and far from the basidiomycete *U. maydis.* However, the intron sequence of *I. japonica* was similar to that of *U. maydis.* These data suggest that these introns are horizontally transmitted.

Some group I introns, including that of *I. japonica*, which lack an endonuclease coding region, are unlikely to be mobile. It is unclear where the ‘‘stable’’ intron came from, or if it has been present from the beginning. Recently, intron transposition by reverse splicing was

proposed, and it did not limit the intron type (Belfort 1993). The intron of *I. japonica* was probably moved by such a process. Further sequence analysis many provide us with the mechanism of the intron mobility.

The distribution of group I introns within fungi is very complicated. In this study the phylogenetic relationships between the intron sequences and the host genome sequences (e.g., 18S rDNA) were not coincident. Therefore, lateral transfer may have played an important role in the evolution of fungal group I introns.

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