Botanical Studies (2009) 50: 137-147.

Group I introns in small subunit ribosomal DNA (SSU-rDNA) of cereal *Phaeosphaeria* species

Chih-Li WANG[1,6], Pi-Fang Linda CHANG[2], Ying-Hong LIN[2], Arkadiusz MALKUS[3], Ling-Yan GAO[4] and Peter P. UENG[5,*]

[1]*Department of Plant Protection, Fengshan Tropical Horticultural Experiment Station, Agricultural Research Institute, Kaohsiung 830, Taiwan*
[2]*Department of Plant Pathology, National Chung Hsing University, Taichung 402, Taiwan*
[3]*Department of Plant Pathology, Plant Breeding and Acclimatization Institute, Radzikow, Poland*
[4]*Inner Mongolia Agriculture University, College of Ecology and Environmental Science, Inner Mongolia*
[5]*Molecular Plant Pathology Laboratory, Plant Science Institute, U.S. Department of Agriculture, ARS, Beltsville, MD 20705, USA*

(Received May 14, 2008; Accepted November 20, 2008)

ABSTRACT. In a study of small subunit ribosomal RNA (SSU-rRNA) gene sequences in cereal and a grass *Phaeosphaeria* species, group I introns were found in 9 of 10 *P. avenaria* f. sp. *avenaria* (Paa) isolates from oat (*Avena sativa* L.), 1 of 2 *Phaeosphaeria* sp. (P-rye) isolates (Sn48-1) from Polish rye (*Secale cereale* L.), 1 *Phaeosphaeria* sp. (P-dg) isolate (S-93-48) from dallis grass (*Paspalum dilatatum* Poir.) and both heterothallic *P. a.* f. sp. *triticea* (Pat2) isolates (ATCC26370 and ATCC26377) from foxtail barley (*Hordeum jubatum* L.). There were no group I introns in wheat- and barley-biotype *P. nodorum* (PN-w and PN-b), homothallic *P. a.* f. sp. *triticea* (Pat1) and *P. a.* f. sp. *triticea* (Pat3) from the state of Washington. Based on the reference 16S rDNA nucleotide sequence of *Escherichia coli* (accession number J01695), the intron-inserted positions of Pav.nS943, Pse.nS943, Ppa.nS1199 and Pho.nS1533 were determined to be at nt943, nt943, nt1199 and nt1533, respectively. The sizes of the introns were 362 bp for Pav.nS943 (from Paa), 363 bp for Pse.nS943 (from P-rye), 460 bp for Pho.nS1533 (from Pat2) and 383 bp for Ppa.nS1199 (P-dg). The intron-inserted position at nt1533 found in SSU-rRNA of Pat2 pathogen was newly discovered. The phylogenetic relationships based on aligned conserved secondary structure component sequences of group I introns showed that three introns from cereal *Phaeosphaeria* species (Pav.nS943, Pse.nS943 and Pho.nS1533) were likely affiliated with subgroup IC1 introns while Ppa.nS1199 intron from the dallis grass pathogen belonged to subgroup IE3.

Keywords: Introns; *Phaeosphaeria*; Phylogenetic relationships; Ribosomal RNA gene; Small subunit; Wheat.

INTRODUCTION

Length polymorphisms in the ribosomal RNA (rRNA) genes are due to DNA fragment insertions, deletions, duplications and the presence of group I introns. Group I introns are one of the major class introns widespread in mitochondria, chloroplasts and nuclear rDNA of eukaryotes including fungi, algae, slime molds and plants, and in eubacteria and protist (Turmel et al., 1991; Haugen et al., 2005). Group I introns are also infrequently reported in mitochondrial genomes of lower sea animals, viruses and phages, and absent in prokaryotes including archaea and bacteria (Lonergan and Gray, 1994; Beagley et al., 1998; Nishida et al., 1998; Riipinen and Alatossava, 2004; Sandegren and Sjöberg, 2004; Fukami et al., 2007; Stankovic et al., 2007). Some group I introns are experimentally proven to act as mobile genetic elements by reverse splicing and site-specific endonuclease restriction (Lambowitz and Belfort, 1993; Saldanha et al., 1993; Belfort and Perlman, 1995; Roman and Woodson, 1998; Haugen et al, 2005).

The insertion positions of group I introns in nuclear SSU-rRNA gene were standardized by using 16S rDNA nucleotide sequence of *Escherichia coli* (Accession number J01695) as reference. Group I introns with the same insertion positions are suggested to have evolved by vertical transmission, along with the occasional loss and horizontal transfer (Nikoh and Fukatsu, 2001; Feau et al., 2007). In vertical transfer, the phylogenetic relationship of introns inserted at the same position is expected to be significantly congruent with the phylogeny of their hosts (Nikoh and Fukatsu, 2001). When the phylogeny of group I introns differ significantly from the host genomes, it is

[6]Present address: Department of Plant Pathology and Microbiology, Texas A & M University, College Station, TX 77840, USA.
*Corresponding author: E-mail: Peter.Ueng@ars.usda.gov; ppuuueng@gmail.com; Tel: +1-301-504-6308; Fax: +1-301-504-5449.

a strong evidence of horizontal transfer (Goddard and Burt, 1999; Holst-Jensen et al., 1999; Simon et al., 2005). The presence of group I introns in rRNA in some fungal species is useful in developing molecular markers for PCR amplification to separate them from other species which had no introns (Neuvèglise et al., 1997; Chen et al., 1998).

In a preliminary survey, length polymorphisms were observed in the small subunit ribosomal RNA (SSU-rRNA) genes of several *Phaeosphaeria* species (Anamorph: *Stagonospora*). The SSU-rRNA gene sequence of *P. avenaria* f. sp. *avenaria* (Paa) AFTOL-ID 280 isolate containing an intron was deposited previously (Accession number AY544725). Characteristics of these length polymorphisms in SSU-rRNA of three cereal and one grass *Phaeosphaeria* species were studied here. This is the first report of a new group I intron inserted at position nt1533 corresponding to the *Escherichia coli* 16S rDNA found in *P. avenaria* f. sp. *triticea* (Pat2) pathogens isolated from foxtail barley (*Hordeum jubatum* L.).

MATERIALS AND METHODS

PCR amplification and sequencing

Procedures for fungal culture in a liquid medium and for genomic DNA (gDNA) isolation were described previously (Ueng et al., 1992). The gDNA was purified by CsCl gradient ultracentrifugation. The primer set NS1 (GTAGTCATATGCTTGTCTC) and NS8 (TCCGCAGGTGCACCTACGGA) designed from the rDNA sequence (nt10138-nt10120 / nt8368-nt8387) of *Saccharomyces cerevisiae* (Accession number Z73326) were used for amplifying partial SSU fragments in strains of *Phaeosphaeria*. *Phaeosphaeria* species including 5 wheat-biotype *P. nodorum* (PN-w), 6 barley-biotype *P. nodorum* (PN-b), 10 *P. avenaria* f. sp. *avenaria* (Paa), 5 homothallic *P. avenaria* f. sp. *triticea* (*P. a.* f. sp. *triticea*, Pat1), 2 heterothallic *P. a.* f. sp. *triticea* from foxtail barley (Pat2), 1 *P. a.* f. sp. *triticea* from the state of Washington (Pat3), 2 *Phaeosphaeria* sp. from Polish rye (P-rye) (Reszka et al., 2006) and 1 from dallis grass (*Paspalum dilatatum* Poir.) (P-dg) were used (Table 1). For examining intron insertion in the large subunit ribosomal RNA (LSU-rRNA) genes of those *Phaeosphaeria* species, primers sets LR0R/LR7 (ACCCGCTGAACTTAAGC/ TACTACCACCAAGATCT), LR7R/LR10 (GCAGATCTT GGTGGTAGTAG/GTCAAGCTCAACAGGGTCTTC) and LR10R/LR13 (GAAGACCCTGTTGAGCTTGAC/ GATCGTAACAACAAGGCTACTC) were used for PCR amplifications. PCR amplification was performed in 50 µl reaction mixtures containing reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH 9.0 at 25°C, 0.1 % Triton X-100), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.5 µM of each primer, 80 ng gDNA, and 1.0 unit of *Taq* DNA polymerase (Promega, Madison, WI). Reaction parameters were: denaturation (94 °C, 3 min) followed by 40 cycles of 94°C (20 s), 55°C (30 s), and 72°C (1 min), and a final incubation step at 72°C (10 min). Isolation and direct sequencing of PCR products were conducted as described previously (Ueng et al., 2003).

Intron splicing in SSU rRNA

To determine the intron splicing in rRNA, the total RNA digested with DNase I was used. Both gDNA and total RNA were isolated from *Phaeosphaeria* cultures grown in YMS (0.5% malt extract, 0.5% yeast extract and 2.0% sucrose) liquid medium with shaking at 125 rpm for 7-14 days at 27°C (Ueng et al., 1992). The gDNA was partially purified, ribonuclease A treated and without CsCl gradient ultracentrifugation (Ueng et al., 1992). Extraction of total RNA mainly followed the protocols previously described (Wang et al., 2007). Lack of residual gDNA in total RNA was evidenced by not being able to amplify the partial histidinol dehydrogenase (*Hdh1*) gene fragment by the primer set 15A/12-1 (ATGCCGGCAGGACCCAGTGA/ CTATCAAGCTACGCCAAGTCGC) and *Taq* DNA polymerase (Unpublished data, Wang et al., 2007). The first strand (1×) cDNA was synthesized with random primers $p(dN)_6$ and the First Strand cDNA Synthesis Kit (Roche Diagnostics Corporation, Indianapolis, IN). PCR amplified fragments amplified from gDNA and 1× cDNA were isolated, sequenced and compared (Ueng et al., 2003). Primers, NS1 and NS8, and other specifically designed ones, such as Fun5 (GACCAGGACTTTTACTTTG), Fun6 (CTGTCAATCCT TATTTCATCTG), Fun8 (GTGTTGAGTCAAATTAAGC) and SR6 (TGTTACGACTTTTACTT), were used for amplifying and sequencing partial SSU-rDNA fragments from gDNA and the first strand cDNA.

Secondary structure modeling of group I introns in pre-rRNA

The secondary structures of group I introns were predicted and constrained according to the expected conserved sequences and structures for subgroup IC (Cech, 1988) and subgroup IE (Suh et al., 1999; Li and Zhang, 2005) by utilizing the 'RNA structure, version 4.4' program (Mathews et al., 1999, 2004) and exported in '.ct' format for further refinement with the 'RnaViz 2' program (De Rijk et al., 2003). The intron nomenclatures followed Johansen and Haugen's proposal (2001) and the structural conventions for group I introns followed Burke et al. (1987) and Gutell (1993).

Subgroup introns by sequence analysis

Regardless of the exon sequences, the insertion positions, and their host phylogenetic relationships, group I introns were divided into 5 major groups (IA-IE), which included 14 subgroups (IA1-AI3, IB1-IB4, IC1-IC3, ID, and IE1-IE3). Most of group I introns in the nuclear SSU-rDNA have been grouped in either IC1 or IE, whilst in the organelle (mitochondria and chloroplast), SSU-rDNA grouped in IA3 and IC2 except for 1 in IB1 (www.rna.icmb.utexas.edu, Cannone et al., 2002). To identify sub groupings of the introns found in the nuclear SSU-rDNA

Table 1. Isolates of *Phaeosphaeria* species used for small subunit ribosomal RNA (SSU-rRNA) gene analysis.

Species	Original host	Year	Geographic location	Intron names and sizes (bp) in SSU	Accession number	
					SSU [a]	LSU [a]
***Phaeosphaeria nodorum* (wheat-biotype) (PN-w)**						
Sn37-1	Wheat (*Triticum aestivum* L.)	-	Szelejewo, Poland	-	EU189213	EF590318
Sn27-1	Wheat	-	Sieradz, Poland	-	(= EU189213)	(= EF590318)
S-81-B13B	Barley (*Hordeum vulgare* L.)	1981	Bledsoe, GA, USA	-	(= EU189213)	(= EF590318)
S-81-W15	Wheat	1981	Sheridan, OR, USA	-	(= EU189213)	(= EF590318)
S-78-13	Wheat	1978	Toluca, Mexico	-	(= EU189213)	(= EF590318)
***Phaeosphaeria* sp. (From Poland) (P-rye)**						
Sn48-1	Winter rye (*Secale cereale* L.)	1995	Jelenia Góra, Poland	Pse.nS943 (363)	EU189214	EF590319
Sn23-1	Winter rye	-	Bydgoszcz, Poland	-	EU189215	(= EF590319)
***Phaeosphaeria nodorum* (barley-biotype) (PN-b)**						
S-80-603	Barley	1980	Williamson, GA, USA	-	(= EU189212)	EF590320
S-80-611 (ATCC200842)	Barley	1980	Laurinburg, NC, USA	-	EU189212	(= EF590320)
S-83-2 (ATCC200841)	Barley	1983	Tifton, GA, USA	-	(= EU189212)	(= EF590320)
S-83-7	Barley	1983	Holland, VA, USA	-	(= EU189212)	(= EF590320)
S-84-2	Barley	1984	Moultrie, GA, USA	-	(= EU189212)	(= EF590320)
S-93-38	Barley	1993	Floyd County, GA, USA	-	(= EU189212)	(= EF590320)
***Phaeosphaeria avenaria* f. sp. *avenaria* (Paa)**						
1920WRS	Oat (*Avena sativa* L.)	2002	Manitoba, Canada	Pav.nS943 (362)	EU189203	
1921WRS	Oat	2002	Manitoba, Canada	Pav.nS943 (362)	(= EU189203)	
1919WRS	Oat	2002	Manitoba, Canada	Pav.nS943 (362)	EU189204	EU223257
Sa37-2	Oat	2001	Radzików, Poland	Pav.nS943 (362)	EU189205	(= EF590321)
ATCC12277	Oat	-	USA	Pav.nS943 (362)	EU189206	EF590321
ATCC58582	Wheat	1984	New York, USA	Pav.nS943 (362)	EU189207	
Saa001NY-85	Oat	1985	New York, USA	Pav.nS943 (362)	(= EU189207)	EU223256
ATCC58583	Wheat	1984	New York, USA	Pav.nS943 (362)	(= EU189207)	
5413	Oat	1983	Ontario, Canada	Pav.nS943 (362)	(= EU189207)	(= EF590321)
Sat002NY-84	Wheat	1984	New York, USA	-	EU189208	
***Phaeosphaeria avenaria* f. sp. *triticea* (Pat1)**						
Sat24-1	Wheat	-	Warmińsko-Mazurskie, Poland	-	EU189209	EF590322
12889	Wheat	1997	Mandan,ND, USA	-	(= EU189209)	(= EF590322)
Sa39-2	Oat	2001	Radzików, Poland	-	(= EU189209)	(= EF590322)
ATCC26374	Foxtail barley (*Hordeum jubatum* L.)	1972	Minnesota, USA	-	(= EU189209)	(= EF590322)
ATCC26375	Foxtail barley	1972	Minnesota, USA	-	(= EU189209)	(= EF590322)
***Phaeosphaeria avenaria* f. sp. *triticea* (Pat2)**						
ATCC26370	Foxtail barley	1972	Minnesota, USA	Pho.nS1533 (460)	EU189210	EF590323
ATCC26377	Foxtail barley	1972	Minnesota, USA	Pho.nS1533 (460)	(= EU189210)	(= EF590323)
***Phaeosphaeria avenaria* f. sp. *triticea* (Pat3)**						
S-81-W10	Wheat	1981	Washington, USA	-	EU189211	EF590324
***Phaeosphaeria* sp. (P-dg)**						
S-93-48	Dallis grass (*Paspalum dilatatum* Poir.)	1993	Griffin, GA, USA	Ppa.nS1199 (383)	EU189216	EF590325

[a]SSU = small subunit; LSU = large subunit.

of *Phaeosphaeria* species, 12 intron nucleotide sequences representative of subgroup IC, IE, IB2 and IA3 were randomly chosen from the alignments made by Michel & Westhof (1990) and Li & Zhang (2005), manually aligned with 4 *Phaeosphaeria* intron nucleotide sequences based on the core secondary structure components P1 to P10, and refined by removing the components P2, P2.1, P7.1, P7.2, and P9.1 to P9.3, which are not presented in all groups (Table 2). The final alignment data were analyzed using Mega Version 4.0 (http://www.megasoftware.net/index.html, Tamura et al., 2007). One thousand data sets analyzed from 167 informative nucleotides sites were generated by bootstrap re-sampling and evaluated by Neighbor-Joint (NJ) method with Jukes-Cantor model.

RESULTS

Identification of the intron sequences in SSU-rDNA

Partial SSU-rDNA fragments amplified from gDNA with the primer set NS1/NS8 showed length variations in several cereal *Phaeosphaeria* species (Figure 1A). There were two DNA fragments produced in P-rye Sn48-1 isolate (Figure 1A, lane 6). Based on the nucleotide sequences, group I introns were identified in the following isolates: 9 of 10 Paa, 1 of 2 P-rye, both Pat2 and 1 P-dg (Table 1). The analysis also showed that group I introns in SSU-rDNA in *Phaeosphaeria* will be removed from the pre-rRNA during rRNA maturation (Figure 1B). Without group I introns, the length of partial SSU fragments amplified by the primer set NS1/NS8 are expected to be 1,769 bp in *Phaeosphaeria* species. Pat1 and Pat3 had the same nucleotide sequences for the partial SSU-rDNA as that in PN-w. Compared to partial SSU-rDNA sequence of PN-w, there is l nucleotide substitution in PN-b, 2 in Paa and Pat2, 3 in P-dg and 4 in P-rye isolates (Data not shown). The sizes of group I intron in SSU-rDNA were as followed: 362 bp in Paa, 363 bp in P-rye, 460 bp in Pat2 and 383 bp in P-dg isolates (Table 1). On the other hand, partial nucleotide sequences of large subunit (LSU-) rRNA gene amplifying with three primers sets LR0R/LR7, LR7R/LR10 and LR10R/LR13 found no intron insertions in those *Phaeosphaeria* species (Accession numbers EF590318-EF590322 and EU223256-EU223257, Table 1).

Intron positions and naming

Based on the 1,542 bp-long reference 16S rDNA sequence of *E. coli* (accession number J01695), the intron positions were determined to be at nt943 for Paa and P-rye, nt1199 for P-dg and nt1533 for Pat2 (Figure 2). Since Paa (*P. avenaria* f.sp. *avenaria*) was first identified as a pathogen from oat [*Avena sativa* L.], the group I intron found in this pathogen was named as Pav.nS943 following Johansen and Haugen's proposal (2001). However, the scientific names and classification of P-rye, Pat2 and P-dg pathogens have not yet been recognized. In this paper, the first two letters of their respective host

Table 2. Alignment of 4 group I introns nucleotide sequences in *Phaeosphaeria* species. Name of the introns followed the nominations proposed by Johansen and Haugen (2001). Vertical arrows in P1 and P10 components indicated the intron-exon junctions. Underlined nucleotides participated in the secondary structures. Number of nucleotides eliminated from the alignment was indicated in parentheses.

Introns	Secondary structure components													
	P1	P1′	P3	P4	P5		P5′	P4′	P6					
Pav.nS943	*CAGGCGT* ↓ AAACGTA	---TAGCTCTGCGCC- (62)	--GAGACCCTTAAATTGCGGGGAATTCGCGAAGCACTCAGCT- (60)	-TTTGTATGTGTT-AATCCGCA-	GCCAAGCCCCTCTATCAAT	------								
Pse.nS943	*CAGGCGT* ↓ AAACGTA	----TAGCTCTGCGCC- (62)	--GAGACCCTTAAATTGCGGGGAATTCGCGAAGCACTCAGCT- (61)	-TTTGTATGTGTT-AATCCGCA-	GCCAAGCTCCTCTATCATT	------								
Pho.nS1533	*CAAGGTT* ↓ GATTCTTCGC-	TTGCGGAAGCCT- (69)	--AACACTTTCGAATTGCGGGGAA-	--CCCCTAAGAGCCTTTT- (64)	-GGTGAAATGGGC-	AATCCGCA-	GCGAAGCCCCTGC	----------						
Ppa.nS1199	*AACAGGT* ↓ TAACCT	----ACACAGGCCTGT- (106)	-AACACCACCTGGTACAGGGAAC	--GCCGGACCCGCTATTGCG	--TATGCGGGTTGGCC-	GATCCTGT-	GGCGAGCTAAGGTA	----------						

(Continued)

Introns	Secondary structure components													
	P6′	P7	P3′	P8	P8′	P7′	P9.0	P9	P9′	P9.0′	P10			
Pav.nS943	GCGAGTGGGGAAGGT-	TCAGAGACTA-	AACAGGGGTC-	GGTTAGAACTATGAAGTACATTCTAGCTT-	AAGATATAGTCC-	-GT-	-GTCACTGCT-	GAAAGGCAGTAGA-	(21)-	AAC-	-G ↓ *GGAGCCTGC*			
Pse.nS943	GCGAGTGGAGAAGGT-	TCAGAGACTA-	GACAGGGGTC-	GGTTAGAATGATGAATTATATTCTAGCTT-	AAGATATAGTCC-	-GT-	-GCTACTGTT-	GAAAGGCAGTAGA-	(21)-	AAC-	-G ↓ *GGAGCCTGC*			
Pho.nS1533	-----	TGGGGAACGT-	TCACAGACTA-	AGTGGAAGTG-	GGTGGGGTATTT-	---	-ACATGCCCTGCTT-	AAGATATAGTCG-	-AG-	-CCCCTTTG-	-GAAACTCTGGGGA-	(59)-	GCT-	GT ↓ *TCCGTAGGT*
Ppa.nS1199	--ACGCTTAGCCGTC-	GCAACGCGCG-	GAAAGGGGTG-	GGTCAACAATA-	----------	AGTTGGCTT-	AAGGTACGTGCT-	AAT-	-CCCTTGG-	---	GTAACCAAGCCTC-	(93)-	AAT-	-G ↓ *CTGTGATGC*

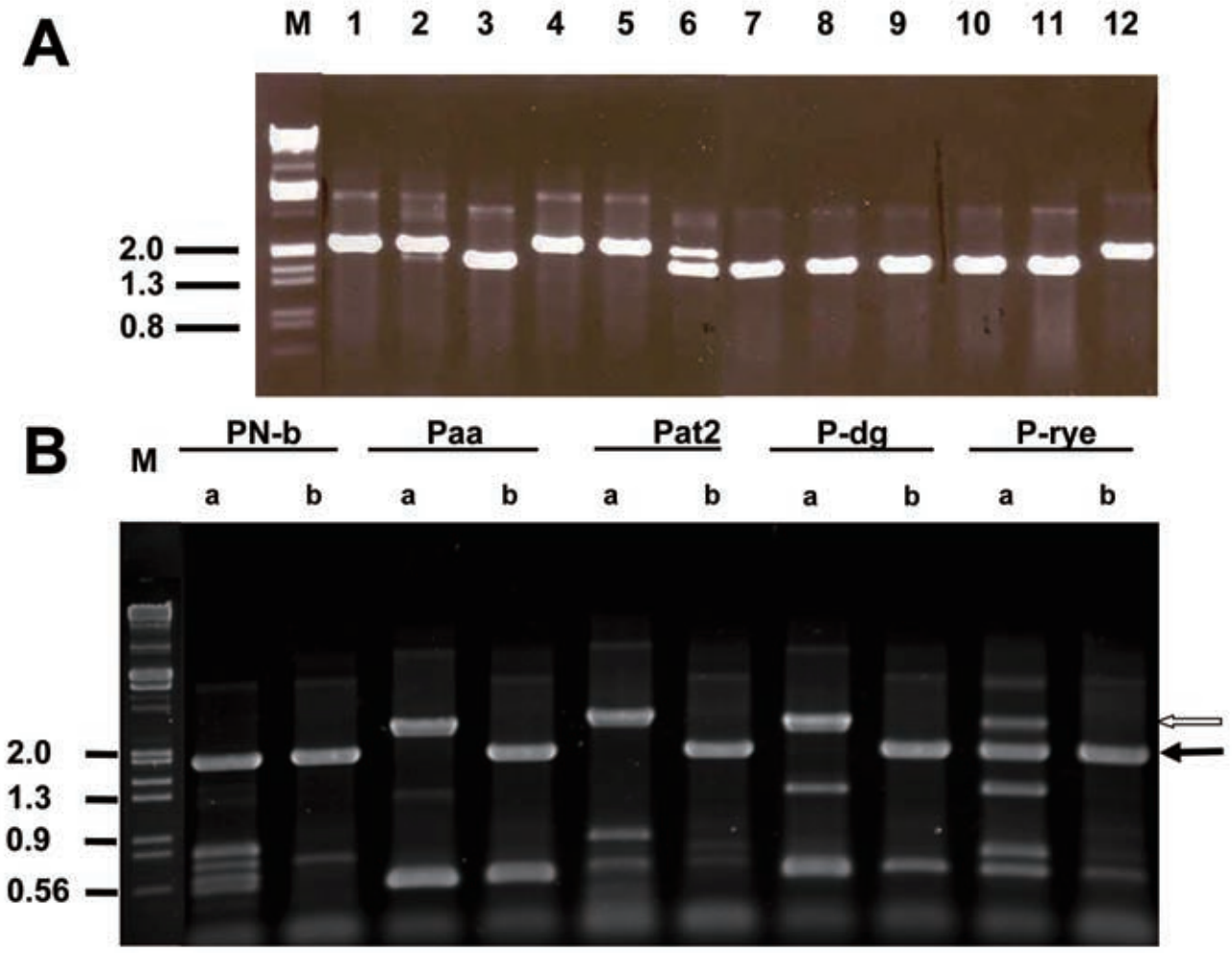

Figure 1. Amplification of partial small subunits of ribosomal DNA (SSU-rDNA) in *Phaeosphaeria* species. A, Partial SSU-rDNA was amplified from the genomic DNA (gDNA) with NS1/NS8 primer set. M = Molecular markers of λ DNA cut with *Hin*dIII and *Eco*RI restriction enzymes. 1-2 = *Phaeosphaeria avenaria* f. sp. *avenaria* (Paa) (ATCC12277, Saa001NY-85); 3 = *P. avenaria* f. sp. *triticea* (*P. a.* f. sp. *triticea*, Pat3) (S-81-W10); 4-5 = Heterothallic *P. a.* f. sp. *triticea* (Pat2) (ATCC26370, ATCC26377); 6 = *Phaeosphaeria* sp. from Polish rye (P-rye) (Sn48-1); 7 = *P. nodorum*, wheat-biotype (PN-w) (Sn27-1); 8-9 = *P. nodorum*, barley-biotype (PN-b) (S-84-2, S-83-2); 10-11 = Homothallic *P. a.* f.sp. *triticea* (Pat1) (Sat24-1, 12889); 12 = *Phaeosphaeria* sp. from dallis grass (P-dg) (S-93-48); B, Partial SSU-rDNA was amplified from the gDNA (a) and the first strand (1×) cDNA (b) with NS1/NS8 primer set. Solid arrows indicated either no introns in gDNA and cDNA or spliced cDNA. Open arrows indicated the presence of introns in SSU-rDNA of gDNA. Isolates used were: PN-b = S-80-611; Paa = ATCC12277; Pat2 = ATCC26370; P-dg = S-93-48; P-rye = Sn48-1.

plant genus names were temporarily used as the 2nd and 3rd letters in the naming of the introns. Therefore, group I introns found in P-rye (from rye [*Secale cereale* L.]), Pat2 (from foxtail barley [*Hordeum jubatum* L.]) and P-dg (from dallis grass [*Paspalum dilatatum* L.]) are called Pse.nS943, Pho.nS1533 and Ppa.nS1199, respectively.

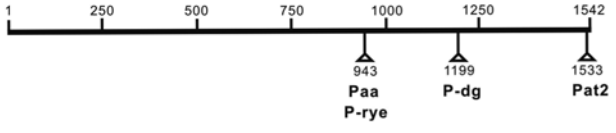

Figure 2. Group I insertions in the small subunit of ribosomal DNA (SSU-rDNA) of *Phaeosphaeria* species. The numbers below each triangle corresponds to the intron positions relative to the SSU-rDNA sequence of *Escherichia coli* (accession number J01695). Paa = *Phaeosphaeria avenaria* f. sp. *avenaria*; P-rye = *Phaeosphaeria* sp. from Polish rye; P-dg = *Phaeosphaeria* sp. from dallis grass; Pat2 = Heterothallic *P. avenaria* f.sp. *triticea*.

Predicted secondary structures of group I intron

The secondary structures of 4 group I introns found in the SSU-rDNA in *Phaeosphaeria* species were composed of nine base-paired segments, characterized by a conserved active structure that was formed by the assembly of the 'J' (including P4-P6) and the 'P' (including P3, P7 - P9)

structural elements, and other peripheral elements needed for splicing (Figure 3) (Burke et al., 1987; Cech, 1988; Michel and Westhof, 1990; Golden et al., 1998; Adams et al., 2004). Internal guide sequences (IGS) were recognized which base pair tightly with the 5′ exon to form a P1 helix before docking into the catalytic core and that permits to stable tertiary interactions during the course of the self-splicing (Been and Cech, 1986; Pyle and Cech, 1991) (Figure 3). Also as expected, a 3′ end exon base ‘U’ immediately upstream of the 5′ intron splicing site and a 3′ end intron base ‘G’ preceding the 3′ intron splicing site were located (Michel and Westhof, 1990). However, there was an exception in Pho.nS1533 intron, which had ‘GU’ preceding the 3’ intron-splicing site (Figure 3C).

Based on 4 consensus sequences (P, Q, R and S elements), Pav.nS943, Pse.nS943 and Pho.nS1533 complied with the expected consensus for groups IA-ID introns, and Ppa.nS1199 with group IE introns (Table 3)

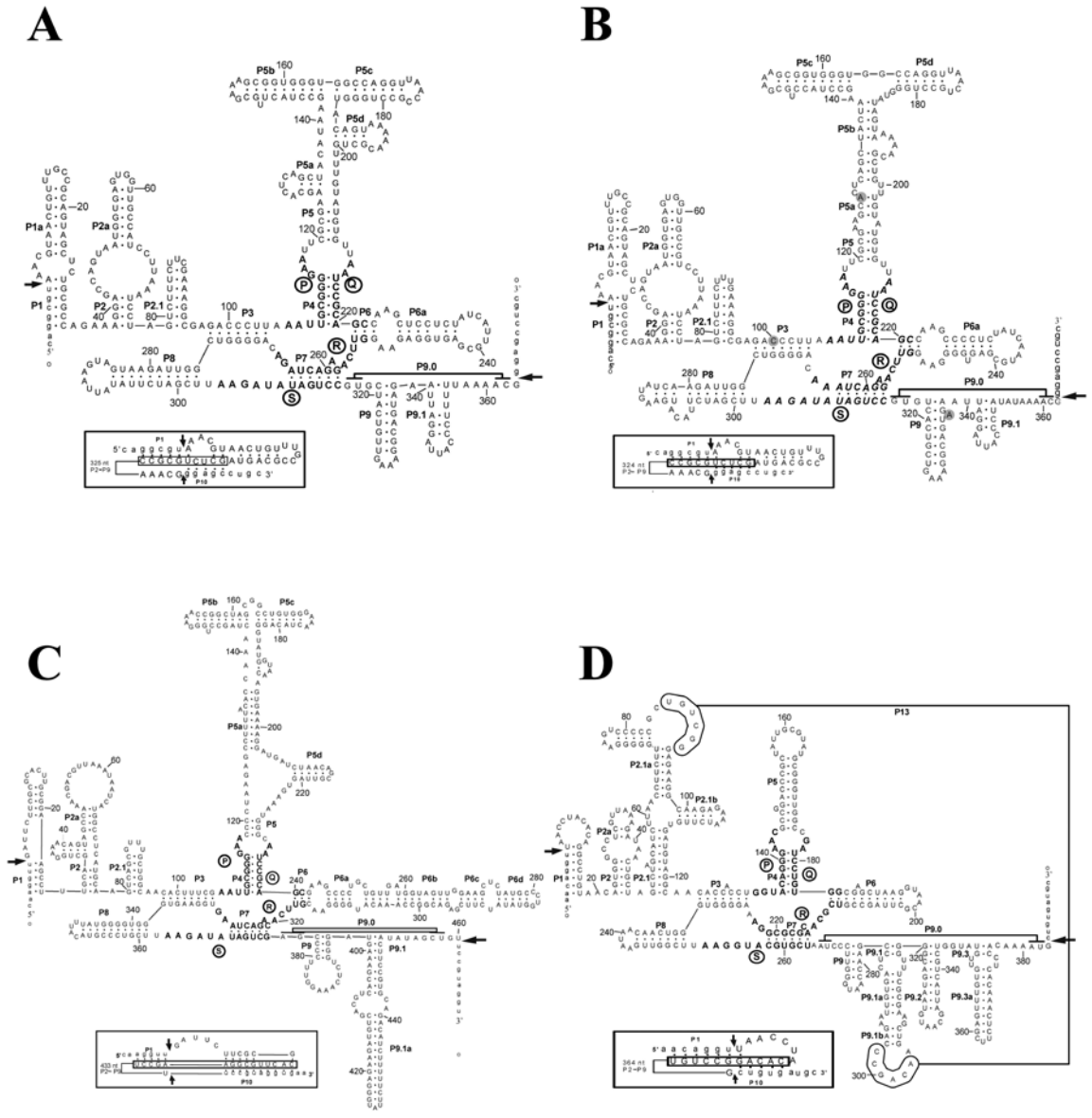

Figure 3. Predicted secondary structure models of *Phaeosphaeria* group I introns. Intron representatives of Pse.nS943 (A), Pav. nS943 (B), Pho.nS1533 (C) and Ppa.nS1199 (D) were shown. The intron sequences were numbered from 5′ end to 3′ end and in uppercase letters whilst the 5′ and 3′ exons in lowercase letters. The bolded and circled letters were the conserved sequence elements, P, Q, R and S. The characteristics of helixes were termed from P1 to P10 following the structural conventions for Group I introns (Burke et al., 1987). The P13 stem formed by base pairing of two remote peripheral elements P2.1 and P9.1 in (D) was indicated. The recognition and binding of a 5′ end internal guide sequences (IGS) with the 3′ exon sequences in splicing were boxed. Three shaded letters in (B) were base substitutions occurred in respective isolates, 1919WRS (at nt100, C → T), Sa37-2 (at nt128, A → G) and ATCC12277 (at nt336, A → G).

(Cech, 1988; Michel and Westhof, 1990; Suh et al., 1999; Gibb and Hausner, 2003; Li and Zhang, 2005). Three introns from cereal *Phaeosphaeria* pathogens (Pav.nS943, Pse.nS943 and Pho.nS1533) had typical long extended P5 domains (P5a-P5d) in their predicted secondary structures (Figure 3A-C). Presence of long extended P5 domains in these introns might suggest that they were ancestral type and able to self-splice (Haugen et al., 2004). Like other group IE introns, there was only a short peripheral P5 region (without P5a-P5d) in the secondary structure of the Ppa.nS1199 intron (Li and Zhang, 2005) (Figure 3D). In the secondary structure of the Ppa.nS1199 intron, formation of a P13 stem by base-pairing of two remote peripheral elements, P2.1 and P9.1, was reported to be an important feature, and play a role in the functional folding of the active structure in subgroup IE intron (Figure 3D) (Li and Zhang, 2005; Xiao et al., 2005).

Subgrouping group I introns

Based on nucleotide sequences of the secondary structure components, phylogenetic relationships of introns from 4 *Phaeosphaeria* with other 11 known group I introns were compared. It appeared that Pav.nS943, Pse.nS943 and Pho.nS1533 introns were closely related to IC1 subgroup, and Ppa.nS1199 belonged to subgroup IE3 (Figure 4).

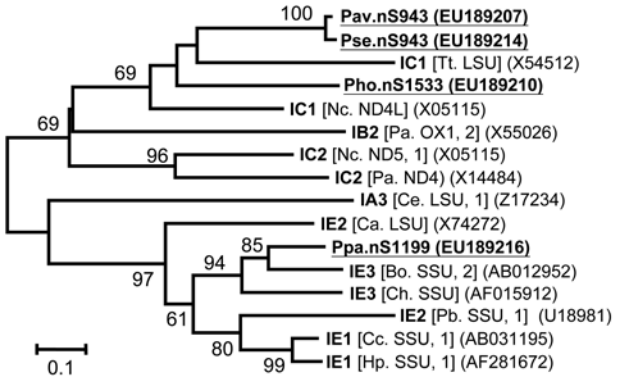

Figure 4. Phylogenetic relationships of group I introns in the small subunit ribosomal DNA (SSU-rDNA) of *Phaeosphaeria* species. Data of aligned secondary structure components from 4 *Phaeosphaeria* introns (see Table 2) and 6 introns representing groups IA-IB and 6 of the group IE aligned respectively by Michel and Westhof (1990) and Li and Zhang (2005) were used for analysis. The numbers in parentheses are GenBank accession numbers. Except for 4 underlined introns from *Phaeosphaeria* species which putatively follows the nomination of Johansen and Haugen's proposal (2001), intron names in square brackets are abbreviated as by Cech (1988). Bootstrap values (with 1000 replications) of the internal branches larger than 50% are indicated.

Table 3. Alignments of 4 consensus sequence elements in the group I introns of ribosomal DNA in *Phaeosphaeria* species.

Introns	Species	Intron size (bp)	Consensus conserved sequences in the elements			
			P	Q	R	S
Groups IA - ID[a]	-	-	au a uncnngaAn ua	c aAunngnag	Guu ga cA GacUana g ccg..ac	c AaGauAuAgUC u
Pav.nS943	Paa	362	aauugcgggggaa	aauccgcagc	guucagagacuaaa	aagauauagucc
Pse.nS943	P-rye	363	aauugcgggggaa	aauccgcagc	guucagagacuaaa	aagauauagucc
Pho.nS1533	Pat2	460	aauugcgggggaa	aauccgcagc	guucacagacuaag	aagauauagucg
Group E[b]			ua g g GG cn gG An Au u a	a c u AUc ng Gg g a a	uc ga ca uc A GCncG gu ac gu	a g c AGG AcguGC c u u
Group E[c]			g ua g gg G cn g An u au u aa	a c u AUc ng Gg g a a	uc ga c ca uc A G ncG gu ac u gu	a g g c AGG Acgu C c u a u
Ppa.nS1199	P-dg	383	gguacagggaac	gauccugugg	ucgcaacgcgcgga	aagguacgugcu

Consensus conserved sequences were after [a]Cech, 1988, [b]Suh et al., 1999 and [c]Gibb and Hausner, 2003.

Underlined nucleotides are proposed to be base-paired in the secondary structure. Bulged nucleotides within base-paired segments are found in #7 nucleotide of the 'R' element. An uppercase letter in consensus conserved sequences designates >90% conservation of the particular nucleotide; a lowercase letter designates 70-90% conservation. A pair of lowercase letters indicates that two nucleotides frequently occupy the position and together account for >90% of the sequences; "n" in a position indicates that no nucleotide is conserved at the level of these criteria. Shaded nucleotides in the group I introns are not identical to consensus sequences.

DISCUSSION

Group I introns are found in both the SSU and LSU rRNA genes in numerous parasitic, lichen-forming and mycorrhizal fungi (De Wachter et al., 1992; Lin et al., 1992; Liu and Leibowitz, 1993; Mercure et al., 1993; Egger et al., 1995; Chen et al., 1996, 1998; Neuvèglise et al., 1997; Tan, 1997; Ito and Hirano, 1999; Myllys et al., 1999; Mavridou et al., 2000; Perotto et al., 2000; Suga et al., 2000; Gibb and Hausner, 2003; Lickey et al., 2003; Wang et al., 2003; Côté et al., 2004). Group I introns were found in the LSU-rDNA of the soil-borne wheat take-all disease pathogen, *Gaeumannomyces graminis* (Tan, 1997). Group I introns were also found in the SSU-rDNA of numerous *Septoria* and other anamorphic species related to the teleomorphic genus *Mycosphaerella* from banana and woody and ornamental plants (Feau et al., 2007). However, the present of group I introns in the rDNA of *M. graminicola* (anamorph: *Septoria tritici*) and *Septoria passerinii*, two important leaf blotch pathogens in cereals, have not been studied.

Allelic heterogeneity of group I introns in rDNA has been reported in numerous fungi. In some organisms, group I introns only inserted in partial repeats of ribosomal DNA repeats tandem located in genome of the same strain. (Lin et al., 1992; Wetzel et al., 1999; Lickey et al., 2003). In strain P-rye Sn48-1, ribosomal repeat regions inserted with intron were amplified by PCR at less frequent than intron-less regions (Figure 1A, lane 6 and Figure 1B, P-rye). The presence of the intron was often variable with strains of a species or among strains of different species. For example, only some strains of *Candida albicans* and *Monilinia fructicola* are reported to have the same group I introns in the rDNA (Mercure et al., 1993; Côté et al., 2004). Also, in the ericoid mycorrhizal fungi, no group I intron was present in all SSU rDNA repeats in 1 of 11 *Oidiodendron maius* isolates (Perotto et al., 2000). The variation in the presence of the introns also found in both Paa and P-rye (Table 1).

Group I introns are widely distributed in nuclear SSU- and LSU-rDNA. With 2,179 introns so far reported in SSU-rDNA, there are 117 intron positions identified in the 1542 bp-long reference *Escherichia coli* 16S rDNA (Accession number J01695) (www.rna.icmb.utexas.edu). Of which 16.5% and 6.2% introns are inserted at the nt943 and nt1199 positions, respectively. In this study, three group I introns from *Phaeosphaeria* (Pav.nS943, Pse.nS943, and Ppa.nS1199) were located in these two common positions (Figure 2). Nevertheless, here we report a new group I intron-inserted position at nt1533 of *E. coli* 16S rDNA, which is identified in Pat2 (Pho.nS1533) (Figure 2).

It had been showed that nucleotide sequences of secondary structure components were conserved in the same subgroups of group I introns which inserted in either nucleus or organelles and even in distantly relative organisms (Michel and Westhof, 1990; Suh et al., 1999). For instance, Pav.nS943, Pse.nS943 and Pho.nS1533 introns were grouped in IC1 with an intron inserted in the nuclear LSU-rDNA of *Tetrahymena thermophila* (Tt. LSU), a ciliated protozoan, rather than other subgroup such as the IE2 in LSU-rDNA of *Candida albicans* (Ca. LSU, X74272), which was more closely related to *Phaeosphaeria* (Figure 4). Furthermore, types of subgroups seem to be associated with the exons in which group I introns inserted. In a collective survey of 747 group I introns from fungal nuclear SSU-rRNA genes, almost all of them belong to either group IC introns (65.6%) or group IE (34.3%) (www.rna.icmb.utexas.edu, Cannone et al., 2002). Four group I introns from *Phaeosphaeria* spp. are also grouped into these two subgroups (Figure 4). Group I introns are commonly inserted in highly conserved regions of the SSU-rRNA genes. It had been shown that the same subgroup group I introns that occupy the same position in SSU-rDNA but in distantly related hosts, tend to share a number of structure features as well as high levels of primary sequence similarities compared to introns at different insertion positions, thus allowing to explore their phylogenetic relationships (Suh et al., 1999; Nikoh and Fukatsu, 2001). It is suggested that the intron insertion positions are related to the nature of the intron subgroups to which they belong. In a comparison with numerous group I introns found in the SSU-rRNA genes from several fungi and green algae, the position at nt1199 tended to be a common position for subgroup IE3, and the position at nt943 for subgroup IC1 (Michel and Westhof, 1990; Takashima and Nakase, 1997). Like the Ppa.nS1199 intron from P-dg, many group I introns inserted at position nt1199 are reported to have group IE secondary structures (Gibb and Hausner, 2003; Li and Zhang, 2005; Feau et al., 2007).

Group I introns were suggested to be either transferred horizontally to the distinct insertion sites or inherited and diverged vertically (Shinohara et al., 1996; Tan, 1997; Mavridou et al., 2000; Gibb and Hausner, 2003, Martín et al., 2003; Wang et al., 2003). Horizontal transfer events were suggested to occur to group I introns of SSU-rDNA in *Septoria* and other anamorphic species related to the teleomorphic genus *Mycosphaerella* (Feau et al., 2007). Based on the Phylogenetic relationships inferred from the β-tubulin (*tubA*), β-glucosidase (*bgl1*), and the second largest subunit of RNA polymerases II (*RPB2*) genes, two sister clades, P-rye-PN-w and Paa-Pat3, were consistently recovered with well supports (Malkus et al., 2005; Reszka et al., 2005; Malkus et al., 2006). An additional intron found in *P. eustoma* isolate AFTOL-ID 1570 (DQ678011), a leaf pathogen of reed manna grass (*Glyceria maxima* (Hartm.) Holmb.), had 99% nucleotide similarity with Pav.nS943 and 93% with Pse.nS943. It is likely that the *Phaeosphaeria* intron inserted at nt943 position was vertically transferred from the common ancestor of *P. eustoma*, Paa, and P-rye, rather than gained from 2 or more horizontal transfer events. During the speciation, intron loss could also have occurred in other *Phaeosphaeria* species nested within the two sister clades such as the P-rye-PN-w and the Paa-Pat3.

LITERATURE CITED

Adams, P.L., M.R. Stahley, A.B. Kosek, J. Wang, and S.A. Strobel. 2004. Crystal structure of a self-splicing group I intron with both exons. Nature **430:** 45-50.

Beagley, C.T., R. Okimoto, and D.R. Wolstenholme. 1998. The mitochondrial genome of the sea anemone *Metridium senile* (Cnidaria): introns, a paucity of tRNA genes, and a near-standard genetic code. Genetics **148:** 1091-1108.

Been, M.D. and T.R. Cech. 1986. One binding site determines sequence specificity of *Tetrahymena* pre-rRNA self-splicing, trans-splicing and RNA enzyme activity. Cell **47:** 207-216.

Belfort, M. and P.S. Perlman. 1995. Mechanisms of intron mobility. J. Biol. Chem. **270:** 30237-30240.

Burke, J.M., M. Belfort, T.R. Cech, R.W. Davies, R.J. Schweyen, D.A. Shub, J.W. Szostak, and H.F. Tabak. 1987. Structural conventions for group I introns. Nucleic Acids Res. **15**: 7217-7221.

Cannone, J.J., S. Subramanian, M.N. Schnare, J.R. Collett, L.M. D'Souza, Y. Du, B. Feng, N. Lin, L.V. Madabusi, K.M. Müller, N. Pande, Z. Shang, N. Yu, and R.R. Gutell. 2002. The comparative RNA web (CRW) site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs. BMC Bioinformatics **3:** 2.

Cech, T.R. 1988. Conserved sequences and structures of group I introns: building an active site for RNA catalysis-a review. Gene **73:** 259-271.

Chen, W., L.E. Gray, and C.R. Grau. 1998. Characterization of a group I intron in the nuclear rDNA differentiating *Phialophora gregata* f.sp. *adzukicola* from *P. gregata* f.sp. *sojae*. Mycoscience **39:** 279-283.

Chen, W., C.A. Shearer, and J.L. Crane. 1996. Identification of a group I intron in the nuclear small subunit rDNA of *Pseudohalonectria lignicola*. Curr. Microbiol. **33:** 84-88.

Côté, M.J., M. Prud'homme, A.J. Meldrum, and M.C. Tardif. 2004. Variation in sequence and occurrence of SSU rDNA group introns in *Monilinia fructicola* isolates. Mycologia **96:** 240-248.

De Rijk, P., J. Wuyts, and R. De Wachter. 2003. RnaViz 2: an improved representation of RNA secondary structure. Bioinformatics **19:** 299-300.

De Wachter, R., J.M. Neefs, A. Goris, and Y. Van de Peer. 1992. The gene coding for small ribosomal subunit RNA in the basidiomycete *Ustilago maydis* contains a group I intron. Nucleic Acids Res. **20:** 1251-1257.

Egger, K.N., G. Osmond, and J.L. Goodier. 1995. Sequence and putative secondary structure of group I introns in the nuclear-encoded ribosomal RNA genes of the fungus *Hymenoscyphus ericae*. Biochimica et Biophysica Acta **1261:** 275-278.

Feau, N., R.C. Hamelin, and L. Bernier. 2007. Variability of nuclear SSU-rDNA group introns within *Septoria* species: incongruence with host sequence phylogenies. J. Mol. Evol. **64:** 489-499.

Fukami, H., C.A. Chen, C.Y. Chiou, and N. Knowlton. 2007. Novel group I introns encoding a putative homing endonuclease in the mitochondrial *cox1* gene of scleractinian corals. J. Mol. Evol. **64:** 591-600.

Gibb, E.A. and G. Hausner. 2003. A group I intron-like sequence in the nuclear small ribosomal subunit gene of the ophiostomatoid fungus *Gondwanamyces proteae*. Mycol. Res. **107:** 1442-1450.

Goddard, M.R. and A. Burt. 1999. Recurrent invasion and extinction of a selfish gene. Proc. Natl. Acad. Sci. USA **96:** 13880-13885.

Golden, B.L., A.R. Gooding, E.R. Podell, and T.R. Cech. 1998. A preorganized active site in the crystal structure of the *Tetrahymena* ribozyme. Science **282:** 259-264.

Gutell, R.R. 1993. Collection of small subunit (16S- and 16S-like) ribosomal RNA structures. Nucleic Acids Res. **21:** 3051-3054.

Haugen, P., H.J. Runge, and D. Bhattacharya. 2004. Long-term evolution of the S788 fungal nuclear small subunit rRNA group I introns. RNA **10:** 1084-1096.

Haugen, P., D.M. Simon, and D. Bhattacharya. 2005. The natural history of group I introns. Trends Genet. **21:** 111-119.

Holst-Jensen, A., M. Vaage, T. Schumacher, and S. Johansen. 1999. Structural characteristics and possible horizontal transfer of group I introns between closely related plant pathogenic fungi. Mol. Biol. Evol. **16:** 114-126.

Ito, Y. and T. Hirano. 1999. A group I intron in the 18S ribosomal DNA from the parasitic fungus *Isaria japonica*. J. Mol. Evol. **48:** 337-340.

Johansen, S. and P. Haugen. 2001. A new nomenclature of group I introns in ribosomal DNA. RNA **7:** 935-936.

Lambowitz, A.M. and M. Belfort. 1993. Introns as mobile genetic elements. Annu. Rev. Biochem. **62:** 587-622.

Li, Z. and Y. Zhang. 2005. Predicting the secondary structures and tertiary interactions of 211 group I introns in IE subgroup. Nucleic Acids Res. **33:** 2118-2128.

Lickey, E.B., K.W. Hughes, and R.H. Petersen. 2003. Variability and phylogenetic incongruence of an SSU nrDNA group I intron in *Artomyces*, *Auriscalpium*, and *Lentinellus* (*Auriscalpiaceae*: Homobasidiomycetes). Mol. Biol. Evol. **20:** 1909-1916.

Lin, H., M.T. Niu, T. Yoganathan, and G.A. Buck. 1992. Characterization of the rRNA-encoding genes and transcripts, and a group-1 self-splicing intron in *Pneumocystis carinii*. Gene **119:** 163-173.

Liu, Y. and M.J. Leibowitz. 1993. Variation and *in vitro* splicing of group I introns in rRNA genes of *Pneumocystic aarinii*. Nucleic Acids Res. **21:** 2415-2421.

Lonergan, K.M. and M.W. Gray. 1994. The ribosomal RNA gene region in *Acanthamoeba castellanii* mitochondrial DNA. A case of evolutionary transfer of introns between mitochondria and plastids? J. Mol. Biol. **239:** 476-499.

Martín, M.P., D.H. Coucheron, and S. Johansen. 2003. Structural features and evolutionary considerations of group IB introns

in SSU rDNA of the lichen fungus *Teloschistes*. Fungal Genet. Biol. **40:** 252-260.

Malkus, A., P.F.L. Chang, S.M. Zuzga, K.R. Chung, J. Shao, B.M. Cunfer, E. Arseniuk, and P.P. Ueng. 2006. RNA polymerase II gene (*RPB2*) encoding the second largest protein subunit in *Phaeosphaeria nodorum* and *P. avenaria*. Mycol. Res. **110:** 1152-1164.

Malkus, A., E. Reszka, C.J. Chang, E. Arseniuk, P.F.L. Chang, and P.P. Ueng. 2005. Sequence diversity of β-tubulin (*tubA*) gene in *Phaeosphaeria nodorum* and *P. avenaria*. FEMS Microbiol. Lett. **249:** 49-56.

Mathews, D.H., J. Sabina, M. Zuker, and D.H. Turner. 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. J. Mol. Biol. **288:** 911-940.

Mathews, D.H., M.D. Disney, J.L. Childs, S.J. Schroeder, M. Zuker, and D.H. Turner. 2004. Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure. Proc. Natl. Acad. Sci. USA **101:** 7287-7292.

Mavridou, A., J. Cannone, and M.A. Typas. 2000. Identification of group-I introns at three different positions within the 28S rDNA gene of the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae*. Fungal Genet. Biol. **31:** 79-90.

Mercure, S., S. Montplaisir, and G. Lemay. 1993. Correlation between the presence of a self-splicing intron in the 25S rDNA of *C. albicans* and strains susceptibility to 5-fluorocytosine. Nucleic Acids Res. **21:** 6020-6027.

Michel, F. and E. Westhof. 1990. Modelling of the three-dimensional architecture of group I catalytic introns based on comparative sequence analysis. J. Mol. Biol. **216:** 585-610.

Myllys, L., M. Källersjö, and A. Tehler. 1999. Variable sizes of introns in the SSU rDNA in three species of *Roccella* (Arthoniales, Euascomycetes). Curr. Genet. **36:** 79-85.

Neuvèglise, C., Y. Brygoo, and G. Riba. 1997. 28S rDNA group-I introns: a powerful tool for identifying strains of *Beauveria brongniartii*. Mol. Ecol. **6:** 373-381.

Nikoh, N., and T. Fukatsu. 2001. Evolutionary dynamics of multiple group I introns in nuclear ribosomal RNA genes of endoparasitic fungi of the genus *Cordyceps*. Mol. Biol. Evol. **18**: 1631-1642.

Nishida, K., S. Suzuki, Y. Kimura, N. Nomura, M. Fujie, and T. Yamada. 1998. Group I introns found in *Chlorella* viruses: biological implications. Virology **242:** 319-326.

Perotto, S., P. Nepote-Fus, L. Saletta, C. Bandi, and J.P.W. Young. 2000. A diverse population of introns in the nuclear ribosomal genes of ericoid mycorrhizal fungi includes elements with sequence similarity to endonuclease-coding genes. Mol. Biol. Evol. **17:** 44-59.

Pyle, A.M. and T.R. Cech. 1991. Ribozyme recognition of RNA by tertiary interactions with specific ribose 2'-OH groups. Nature **350:** 628-631.

Reszka, E., E. Arseniuk, A. Malkus, K.R. Chung, N.R. O'Neill, Q.J. Song, and P.P. Ueng. 2006. A new biotype of *Phaeosphaeria* sp. of uncertain affinity causing Stagonospora leaf blotch disease in cereals in Poland. Plant Dis. **90:** 113.

Reszka, E., K.R. Chung, A. Tekauz, A. Malkus, E. Arseniuk, J.M. Krupinsky, H. Tsang, and P.P. Ueng. 2005. Presence of β-glucosidase (*bgl1*) gene in *Phaeosphaeria nodorum* and *Phaeosphaeria avenaria* f.sp. *triticea*. Can. J. Bot. **83:** 1001-1014.

Riipinen, K.A. and T Alatossava. 2004. Two self-splicing group I introns interrupt two late transcribed genes of prolate-headed *Lactobacillus delbrueckii* phage JCL1032. Arch. Virol. **149:** 2013-2024.

Roman, J. and S. A. Woodson. 1998. Integration of the Tetrahymena Group I intron into bacterial rRNA by reverse splicing in vivo. Proc. Natl. Acad. Sci. USA **95:** 2134-2139.

Saldanha, R., G. Mohr, M. Belfort, and A.M. Lambowitz. 1993. Group I and group II introns. The FASEB J. **7:** 15-24.

Sandegren, L. and B.M. Sjöberg. 2004. Distribution, sequence homology, and homing of group I introns among T-even-like bacteriophages. J. Biol. Chem. **279:** 22218-22227.

Shinohara, M.L., K.F. LoBuglio, and S.O. Rogers. 1996. Group-I intron family in the nuclear ribosomal RNA small subunit genes of *Cenococcum geophilum* isolates. Current Genetics **29:** 377-387.

Simon, D., J. Moline, G. Helms, T. Friedl, and D. Bhattacharya. 2005. Divergent histories of rDNA group I introns in the lichen family Physciaceae. J. Mol. Evol. **60:** 434-446.

Stankovic, S., B. Soldo, T. Beric-Bjedov, J. Knezevic-Vukcevic, D. Simic, and V. Lazarevic. 2007. Subspecies-specific distribution of intervening sequences in the *Bacillus subtilis* prophage ribonucleotide reductase genes. Syst. Appl. Microbiol. **30:** 8-15.

Suga, H., K. Oyabu, M. Ito, K. Kageyama, and M. Hyakumachi. 2000. Detection of intron-like sequences in the small subunit rDNA 3' region of *Fusarium solani*. Mycol. Res. **104:** 782-787.

Suh, S-O., K.G. Jones, and M. Blackwell. 1999. A group I intron in nuclear small subunit rRNA gene of *Cryptendoxyla hypophloia*, an ascomycetous fungus: Evidence for a new major class of group I introns. J. Mol. Evol. **48:** 493-500.

Takashima, M. and T. Nakase. 1997. A phylogenetic analysis of three group I introns found in the nuclear small subunit ribosomal RNA gene of the ballistoconidiogenous anamorphic yeast-like fungus *Tilletiopsis flava*. Genes Genet. Syst. **72:** 205-214.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:** 1596-1599.

Tan, M.K. 1997. Origin and inheritance of group I introns in 26S rRNA genes of *Gaeumannomyces graminis*. J. Mol. Evol. **44:** 637-645.

Turmel, M., J. Boulanger, M.N. Schnare, M.W. Gray, and C. Lemieux. 1991. Six group I introns and three internal transcribed spacers in the chloroplast large subunit ribosomal RNA gene of the green alga *Chlamydomonas*

eugametos. J. Mol. Biol. **218:** 293-311.

Ueng, P.P., G.C. Bergstrom, R.M. Slay, E.A. Geiger, G. Shaner, and A.L. Scharen. 1992. Restriction fragment length polymorphisms in the wheat glume blotch fungus, *Phaeosphaeria nodorum*. Phytopathology **82:** 1302-1305.

Ueng, P.P., E. Reszka, K.R. Chung, E. Arseniuk, and J.M. Krupinsky. 2003. Comparison of glyceraldehyde-3-phosphate dehydrogenase genes in *Phaeosphaeria nodorum* and *P. avenaria* species. Plant Pathol. Bull. **12:** 255-268.

Wang, C., Z. Li, M.A. Typas, and T.M. Butt. 2003. Nuclear large subunit rDNA group I intron distribution in a population of *Beauveria bassiana* strains: phylogenetic implications. Mycol. Res. **107:** 1189-1200.

Wang, C.L., A. Malkus, S.M. Zuzga, P.F.L. Chang, B.M. Cunfer, E. Arseniuk, and P.P. Ueng. 2007. Diversity of the trifunctional histidine biosynthesis gene (*his*) in cereal *Phaeosphaeria* species. Genome **50:** 595-609.

Wetzel, H.C., S.H. Hulbert, and N.A. Tisserat. 1999. Molecular evidence for the presence of *Ophiosphaerella narmari* n. comb., a cause of spring dead spot of Bermuda grass, in North America. Mycol. Res. **103:** 981-989.

Xiao, M., T. Li, X. Yuan, Y. Shang, F. Wang, S. Chen, and Y. Zhang. 2005. A peripheral element assembles the compact core structure essential for group I intron self-splicing. Nucleic Acids Res. **33:** 4602-4611.

*Phaeosphaeria*屬穀類葉枯病菌核糖體小次單元基因之 Group I introns

王智立[1] 張碧芳[2] 林盈宏[2] Arkadiusz MALKUS[3] 高凌岩[4] 翁 溥[5]

[1] 行政院農業試驗所 鳳山熱帶園藝試驗分所植物保護系

[2] 國立中興大學 植物病理學系

[3] 波蘭植物育種及環境適應所 植物病理系

[4] 內蒙古農業大學 生態環境學院

[5] 美國農業部農業研究服務署 植物科學組

本研究報導數種 *Phaeosphaeria* 屬葉枯病菌的核糖體小次單元 (ribosomal small subunit) 基因序列中含有 Group I introns，分別存在於 9 個 *P. avenaria* f. sp. *avenaria* (Paa) 分離株、1 個波蘭裸麥之 *Phaeosphaeria* sp. (P-rye) 分離株、1 個達利雀稗 (dallis grass) 之 *Phaeosphaeria* sp. (P-dg) 分離株及 2 個 *P. a.* f. sp. *triticea* (Pat2) 分離株 ；但未存在於小麥生物型 (wheat-biotype) 及大麥生物型 (barley-biotype) 之 *P. nodorum* (PN-w 及 PN-b) 分離株、同絲型 (homothallic) 之 *P. a.* f. sp. *triticea* (Pat1) 分離株以及 *P. a.* f. sp. *triticea* (Pat3) 分離株。依據寄主植物的屬名，並以大腸桿菌 (*Escherichia coli* J01695) 16S 核糖體 去氧核糖核酸 (rDNA) 序列為 Group I introns 於核糖體小次單元中插入位置的參考座標，將 Group I introns 命名，其名稱及序列大小於上述各病原菌中依序分別為 Pav.nS943-362 bp (Paa)、Pse.nS943-363 bp (P-rye)、Pho.nS1533 -460 bp (Pat2) 及 Ppa.nS1199-383 bp (P-dg)。其中 Pho.nS1533 於第 1533 個核甘酸 (nucleotide) 的插入位置為本文首次報導。進一步根據二級結構的組成，將 Group I introns 序列排序 (alignment)，分析其譜系關係 (phylogenetic relationships)，發現存在於穀類葉枯病菌的 Pav.nS943、Pse.nS943 及 Pho.nS1533 可歸屬於 IC1 亞群，然而存在達利雀稗葉枯病菌的 Ppa.nS1199 則歸屬於 IE3 亞群。

關鍵詞 ： 內含子 ；*Phaeosphaeria*；譜系關係 ； 核糖體核糖核酸基因 ； 小次單元 ； 小麥。