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# Molecular Characterization of the Ribosomal Protein L25 from the Stem Rust Fungus

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## ABSTRACT

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A cDNA clone (pCRL130) encoding a 25S rRNA-binding protein (L25) was isolated from the oat stem rust fungus, *Puccinia graminis* f. sp. *avenae*. The cDNA clone contains a 660 bp insert which encodes a putative polypeptide of 158 amino acids and is highly similar to those of eukaryotic ribosomal L25 proteins. Transcripts of the *P. graminis* L25 gene were detected as early as 48 h after host plants were inoculated with the fungus. Temporal patterns of transcript accumulation were closely correlated with fungal growth in host plants, giving more abundant transcript accumulation with compatible than with incompatible or inappropriate isolates of the fungus. Phylogenetic analysis of L23/L25 sequences divided the basidiomycetes into three well-supported lineages (Urediniomycetes, Hymenomycetes and Ustilaginomycetes) which is consistent with the current taxonomic division, based on ribosomal rDNA sequences. Similar results were obtained for taxonomic divisions among selected ascomycetes samples. This represents the first non-ribosomal RNA gene analysis to confirm the division of basidiomycetes into three lineages and indicates that the L23/L25 amino acid sequence is a useful tool for studying fungal phylogenetics.

Key words : *Puccinia graminis*, Stem rust fungus, Ribosomal protein L25, rRNA-binding protein, Phylogenetic, Basidiomycota, Basidiomycetes

## INTRODUCTION

As part of an investigation of genes involved in host-parasite interaction in oat stem rust (*Avena sativa* and *Puccinia graminis* f. sp. *avena*) [11, 12], a fungal cDNA with high homology to L23/L25 ribosomal protein genes was isolated. L25 and its prokaryotic L23 counterpart are among the 50 to 80 different proteins that comprise ribosomes, depending on the species [32]. L25 and L23 are among the first components to be assembled into large or small subunit ribosomes which occurs in the nucleus, before the subunits are incorporated into mature ribosomes in the cytoplasm. L25 directly interacts with 25S or 28S rRNA of eukaryotes; L23 with 23S rRNA of chloroplasts or prokaryotes [4, 16]. Stepwise dissociation of yeast 60S ribosomal subunits *in vitro* by LiCl revealed that L25 is the last protein dissociated from the large subunit, indicating that L25 is one of the core proteins required for insuring structural integrity and biological function of ribosomes[4]. Functional domains of L25 were shown in yeast to be required for pre-RNA processing steps[29]. Results of recent studies also suggest that L23 of *E. coli* plays a role in the targeting of secretory proteins as an attachment site for the signal recognition particle that binds to newly synthesized polypeptides [28].

Ribosomal genes are necessarily expressed in growing organisms and have been characterized from a diverse range of organisms including animals, plants, fungi (including yeasts), eubacteria and archaebacteria [5, 6, 14, 15, 22]. In fungi, DNA sequences that code for proteins homologous to L23/L25 have been characterized from genomic DNA and expressed RNA sequences for several yeasts, filamentous Ascomycota, and filamentous Basidiomycota (see Table 1). Within the Basidiomycota, homologs of L23/L25 have been characterized for six species (Table 1), including one rust

fungus, *Puccinia triticina* [26]. Since few genes of any type have been cloned from rust fungi and their expression investigated, we report here the isolation and characterization of L25 cDNA isolated from the oat stem rust fungus *Puccinia graminis* f. sp. *avenae*. Presented here are the nucleotide sequence, copy number, and temporal patterns of transcript accumulation in inoculated leaves. Based on deduced amino acid sequences, we also show the presence of a nuclear localization signal and a motif implicated in binding L25 proteins to rRNA. The relatedness of L25 proteins from rust to L23/L25 proteins from other organisms is indicated by phylogenetic analysis. This analysis indicates that L23/L25 amino acid sequences are useful for delineating the major subgroups within the Basidiomycota.

Table 1. Amino acid sequences used for phylogenetic analyses of L23/L25 ribosomal proteins.

| Species | Code | Type of organism | Source |
|---|---|---|---|
| Eukaryotes | | | |
| Fungi | | | |
| Basidiomycota | | | |
| *Puccinia graminis* | Pug | Plant pathogen | Swiss-Prot No. P51997 |
| *Puccinia triticina* | Put | Plant pathogen | GenBank No. BU672714 [a] |
| *Ustilago maydis* | Usm | Plant pathogen | Locus No. UM05998.1 [b] |
| *Coprinus cinereus* | Coc | Mushroom | Contig No. 1.181 [c] (23,000-24,5000 bp) |
| *Pleurotus ostreatus* | Plo | Mushroom | Locus No. AT004881 |
| *Paxillus involutus* | Pai | Ectomycorrhiza | GenBank No. CD271494 [a] |
| *Cryptococcus neoformans* | Crn | Human pathogen | Contig No. 1.59 [d] (182,000-183,000 bp) |
| Ascomycota | | | |
| *Fusarium graminearum* | Fug | Plant Pathogen | Locus No. FG02493.1 [e] |
| *Magnaporthe grisea* | Mag | Plant Pathogen | Locus No. MG10185.4 [f] |
| *Neurospora crassa* | Nec | Filamentous | Locus No. NCU06226.1 [g] |
| *Aspergillus nidulans* | Asn | Filamentous | Locus No. AN8856.2 [h] |
| *Schizosaccharomyces pombe* | Scp | Yeast | Swiss-Prot No. Q10330 |
| *Pichia jadinii* | Pij | Yeast | Swiss-Prot No. P08792 |
| *Saccharomyces cerevisiae* | Sac | Yeast | Swiss-Prot No. P04456 |
| *Kluyveromyces lactis* | K11 | Yeast | Swiss-Prot No. P48045 |
| Mammals | | | |
| *Homo sapiens* | Hos | Human | GenBank No. AAC51934 |
| *Rattus norvegicus* | Ran | Rat | Swiss-Prot No. P29316 |
| Plant | | | |
| *Nicotiana tabacum* | Nit | Tobacco | Swiss-Prot No. Q07761 |
| Worm | | | |
| *Caenorhabditis elegans* | Cae | Nematode | Swiss-Prot No. P48162 |
| Prokaryotes | | | |
| *Methanococcus vannielii* | Mev | Archaebacterium | GenBank No. CAA68741 |

[a] Derived from translation of EST sequence data.
[b] *Ustilago maydis* (http://www.broad.mit.edu).
[c] Derived from translation of *Coprinus cinereus* DNA sequence (http://www.broad.mit.edu).
[d] Derived from translation of *Cryptococcus neoformans* DNA sequence (http://www.broad.mit.edu).
[e] *Fusarium graminearum* (http://mips.gsf.de).
[f] Derived from translation of *Magnaporthe grisea* DNA sequence (http://www.broad.mit.edu).
[g] *Neurospora crassa* (http://www.broad.mit.edu).
[h] *Aspergillus nidulans* (http://www.broad.mit.edu).

## MATERIALS AND METHODS

### Plant and fungal materials

Isolates of stem rust fungi used were *Pga*-6A (CRL culture number 59NE06, race 6A) and *Pga*-1H of race 1H (CRL culture number PGR6812, race 1H) of *Puccinia graminis* f. sp. *avenae*, and *Pgt*-8D (CRL culture number 74-36-924-A, race *Pgt*-SCM) and *Pgt*-4A (CRL culture number 78-21-BB463, race *Pgt*-DFBL) of *P. graminis* f. sp. *tritici* [11]. Urediniospore stock cultures of the isolates were derived from three cycles of single pustule isolation, and maintained as described[19]. Oat cultivar, CI9319, a line derived from Rodney with the *Pg*-2 resistance gene was used. This oat cultivar is compatible (susceptible) with isolate *Pga*-6A giving infection type 4, incompatible (resistant) with *Pga*-1H giving infection type 1, and inappropriate with *Pgt*-8D and *Pgt*-4A giving infection type 0 [11]. Inoculation of eight-day-old seedlings with each isolate was performed as described by Lin *et al*. [11]

### DNA sequencing

pCRL130 was isolated by differentially screening a cDNA library from rust-infected oat for genes preferentially expressed in compatible vs. incompatible host-parasite interactions [11]. pCRL130 was sequenced by the double-stranded dideoxy chain termination method using a DNA sequencing kit (Sequenase Version 2.0, USB, Cleveland, OH). Both strands of the cDNA insert were sequenced. DNA sequence data were analyzed with the GCG package (Wisconsin Package Version 10.0, Genetics Computer Group, Inc. Madison, Wisconsin). Both the nucleotide sequence and the deduced amino acid sequence had been submitted to the GenBank library as accession number U44800 and P51997, respectively.

### Genomic DNA extraction and DNA gel blot analysis

Genomic DNA of the rust fungi was extracted as described by Liu *et al*. [13]. Approximately 5 $\mu$g of genomic DNA per lane was digested with different restriction enzymes, electrophoresed in 0.8% (w/v) agarose gels, and transferred to a nylon filter (Nytran; Schleicher & Schuell, Inc., NH). The filter was then hybridized to $^{32}$P-labeled pCRL130 according to manufacturer's instructions. The probe was labeled using a Random Primer DNA Labeling Kit (GIBCO-BRL, Life Technologies, Inc. Gaithersburg, MD). After hybridization, the filter was rinsed with 1X SSPE/0.1% SDS and then washed in 1X SSPE/0.1% SDS at 42℃ for 15 min, twice in 1X SSPE/0.1% SDS at 60℃ for 15 min each. For higher stringency, an additional wash was carried out in 0.1X SSPE/0.1% SDS at 60℃ for 60 min. Autoradiography was performed at -80℃ for 16 h, with an intensifying screen.

### RNA extraction and RNA gel blot analysis

Total RNA was isolated from oat plants infected with various isolates of the stem rust fungi according to Lin *et al*. [11]. Approximately 30 $\mu$g of total RNA per lane was denatured and electrophoresed in 1.2% (w/v) agarose gels containing formaldehyde, transferred to a nylon filter (Nytran; Schleicher & Schuell, Inc., NH), and hybridized to the $^{32}$P-labeled pCRL130 cDNA insert according to manufacturer's instructions. The probe was labeled using a Random Primer DNA Labeling Kit (GIBCO-BRL, Life Technologies, Inc. Gaithersburg, MD). After hybridization, the filter was rinsed with 1X SSPE/0.1% SDS and then washed in 1X SSPE/0.1% SDS at 42℃ for 15 min, twice in 1X SSPE/0.1% SDS at 60℃ for 15 min each. Verification of the equal amount of RNA loaded per lane was obtained by hybridizing the same filter with 28S subunit ribosomal DNA probe from maize.

### Phylogenetic analysis

Amino acid sequences of selected L23/L25 ribosomal proteins (Table 1) were aligned using the program Clustal X [27] and manually adjusted to obtain optimal alignment (MacVector, Accelrys Inc., Madison, WI). Phylogenetic analysis was performed using the computer program PAUP, version 4.0b10 [25]. To test robustness of the tree branches, Bootstrap analysis was performed using 1000 random data sets. Human, rat, tobacco, worm and bacterial sequences were included as outgroups. The bacterial L23 sequence from *Methanococcus vannielii* was used as a root for the phylogenetic tree.

## RESULTS and DISCUSSION

### Isolation and characterization of a cDNA clone homologous to the ribosomal protein L25

In an attempt to isolate genes that might be involved in pathogenicity of the oat stem rust fungus, *Puccinia graminis* f. sp. *avenae*, a cDNA clone that was found in a compatible but not in an incompatible host-pathogen interaction was isolated by differential hybridization and designated as pCRL130 [10]. DNA blot analysis revealed that pCRL130 hybridized with genomic DNA from *Puccinia graminis* but not with DNA from oat, indicating that pCRL130 is of fungal origin instead of plant (data not shown). DNA sequence analysis revealed that the cDNA was 660 bp in length, contains a single open reading frame including start and stop codons, a putative polyadenylation signal (AATCAAA), and a short polyA tail (Fig. 1). The 660 bp cDNA insert is smaller than the size of the transcript, estimated to be approximately 900 bp (data not shown), and indicates that this cDNA clone lacks a complete 5' non-coding region and polyA tail. The open reading frame encodes a predicted polypeptide of 158 amino acids with a calculated molecular weight of 17.7 kD and a pI value of 11.7. The predicted protein is highly

hydrophilic and its hydropathy pattern is very similar to those of other L23/L25 proteins [33]. Lysine residues make up 14% of the *P. graminis* L25 protein, which is similar to the 15-20% found in other L23/L25 proteins [8, 9, 15, 22, 33, 35]. High lysine content is a characteristic of most ribosomal proteins [31].

Alignment of the putative protein sequence of *P. graminis* L25 with those of L23/L25 proteins (Fig. 2) revealed an extended N-terminal region of approximately 60 to 80 amino acid residues, which is present in *P. graminis* and other eukaryotic L23/L25 proteins, but absent in bacterial and chloroplast L23. In *P. graminis* L25, this segment contains a single nuclear localization signal (NLS), KKAVVK (Fig. 2, 31-36 aa) [21]. This is identical or similar to NLS motifs which are found in the N-terminal region of L23/L25 proteins of other eukaryotic organisms. The NLS is required for import of L23/L25 into the nucleus [21]. Yeasts contain a second NLS motif, KKALK (Fig. 2, 41-46 aa) [21]. Both motifs consist of lysine-lysine-alanine followed by one or two hydrophilic residues and a further lysine residue. Multiple NLS motifs have been found in various nuclear proteins other than L23/L25 proteins [17].

The L25 protein from *P. graminis* contains the motif, KKAF/YVRL (Fig. 2, Motif #3, 141-147 aa). This motif is one of several structural elements [located within amino acid residues 62 to 126 in yeast[20] ] required for binding of L23/L25 protein to Domain III of 23S, 26S or 28S rRNA during early assembly of large ribosomal subunits [5, 7, 15, 30]. This region is highly conserved in L23/L25 proteins of eukaryotic organisms as well as L23 proteins from prokaryotic organisms and chloroplasts [18] (Fig. 2). However, we do not have direct evidence for involvement of motif #3 of *P. graminis* in binding to domain III.

The L25 clone from *P. graminis* (Pug in Table 1 and Fig. 2) has high sequence homology with a L25 clone isolated by Thara *et al.* (2003) from the leaf rust fungus, *P. triticina* (Put in Table 1 and Fig. 2). The two differ at only five deduced amino acid sites. However, the leaf rust sequence is truncated at both ends (Fig. 2) and therefore may have additional differences.

## DNA blot analysis

To estimate the copy number of ribosomal protein L25 genes among different isolates of *P. graminis*, DNA blot analysis was performed with genomic DNA from isolates *Pga*-6A and *Pga*-1H of *P. graminis* f. sp. *avenae* as well as isolate *Pgt*-4A of *P. graminis* f. sp. *tritici*, using the pCRL130 cDNA insert as a probe. Genomic DNA was digested with five restriction enzymes that have no recognition site within the cDNA insert of pCRL130. As shown in Fig. 3, pCRL130 hybridized to a single restriction fragment in each lane, except for DNA from isolate *Pga*-6A digested with *Eco*RI or *Pst*I where two restriction fragments were hybridized. Some variation in the size of the hybridizing restriction were fragments was observed among the three isolates. No change in hybridization pattern or intensity was detected using high vs. low stringency wash conditions (data not shown). This data suggests that *P. graminis* has only one copy of the L25 gene per genome. The additional hybridizing restriction fragments in isolate *Pga*-6A probably relate to heterozygosity between the two genomes of the dikaryotic rust fungus. The genomic DNA used in the blot analyses was isolated from germinating urediniospores, which contain two nuclei derived from haploid mycelium of differing mating types. Heterozygosity between the paired nuclei of rust fungus dikaryons has been demonstrated with DNA markers [36] as well as isozyme markers [3].

In *Saccharomyces cerevisiae* and most bacteria studied so far a single copy of the L25 gene is found [8, 9, 35]. However, chloroplasts of wheat and tobacco and most eukaryotes contain multiple copies of the L25 gene [2, 15, 22, 33, 34]. In mammalian cells, only one copy of the L25 genes is functional; the other copies are pseudogenes [32]. For wheat chloroplasts, two copies of L23 genes are assumed to be functional and the third copy is not [2].

## L25 transcript accumulation

To study the accumulation patterns of *P. graminis* L25

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CAGGAACTCAAGACTCGACATGCCGCCAAAATCATCGACCAAGGCCGA  48
                   M  P  P  K  S  S  T  K  A  E

ACCTAAGGCCTCATCGGCCAAGACCCAAGTGGCCAAGGCCAAATCGGC  96
 P  K  A  S  S  A  K  T  Q  V  A  K  A  K  S  A

CAAGAAGGCGGTCGTCAAGGGTACCTCCTCCAAAACTCAGCGTCGTAT 144
 K  K  A  V  V  K  G  T  S  S  K  T  Q  R  R  I

TCGCACTTCGGTCACCTTCCGAAGACCCAAGACTCTCCGACTATCCCG 192
 R  T  S  V  T  F  R  R  P  K  T  L  R  L  S  R

AAAGCCGAAATACCCCAGAACTTCGGTCCCACATGCTCCTCGAATGGA 240
 K  P  K  Y  P  R  T  S  V  P  H  A  P  R  M  D

TGCCTACCGGACATTGGTCCGTCCTTTGAACACCGAGAGCGCCATGAA 288
 A  Y  R  T  L  V  R  P  L  N  T  E  S  A  M  K

GAAAATCGAAGACAACAACACTCTTCTCTTCATTGTTGATTTGAAGGC 336
 K  I  E  D  N  N  T  L  L  F  I  V  D  L  K  A

TAACAAGCGACAAATTGCCGATGCCGTCAAGAAACTTTATGACGTTAC 384
 N  K  R  Q  I  A  D  A  V  K  K  L  Y  D  V  T

ACCCCTTCGTGTGAACACCCTCATTCGTCCCGACGGCAAAAAGAAAGC 432
 P  L  R  V  N  T  L  I  R  P  D  G  K  K  K  A

TTTTGTTCGTCTGACCCCTGAAGTTGATGCCTTAGACATTGCTAACAA 480
 F  V  R  L  T  P  E  V  D  A  L  D  I  A  N  K

GATCGGTTTCATCTAAAGATCTGCTATCACGTGGTTGGGTTGAGGGTG 528
 I  G  F  I  *

ATCGAATCGGGTTTGTGCTCTCAGCCAGTTCCCGTCGAGGTTCAGCAA 576
CTATGCATTGTAATGACGATTCTTATAGCTACATGTCAAATCAAATCT 624
ATGGCTCATTCTCTACCACTAAAAAAAAAAAAAAAA             660
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Fig. 1. Nucleotide sequence of cDNA clone pCRL130 from *P. graminis* f.sp. *avenae* encoding a L25 ribosomal protein. Single letter code for the predicted amino acid sequence is indicated below the DNA sequence. The asterisk denotes the translational stop codon. A potential polyadenylation signal is indicated by a box. The nucleotide sequence has been submitted to the GenBank library as accession number U44800.

transcripts during fungal growth in infected oats, 8-day-old seedling leaves were either mock-inoculated with mineral oil, or inoculated with compatible isolate *Pga*-6A, incompatible isolate *Pga*-1H and inappropriate isolate *Pgt*-8D and sampled from 16 to 72 h after inoculation (AI). Accumulation of L25 transcripts in the samples was determined by RNA blot analysis (Fig. 4) using the pCRL130 cDNA insert as a probe. With the compatible isolate (*Pga*-6A), traces of transcript were present at 36 and 42 h AI, but transcripts were first clearly evident at 48 h AI. Transcript became highly abundant by 72 h AI. As shown by Lin *et al*. (1998), the compatible isolate produced colonies 120 $\mu$m long at 48 h AI and highly

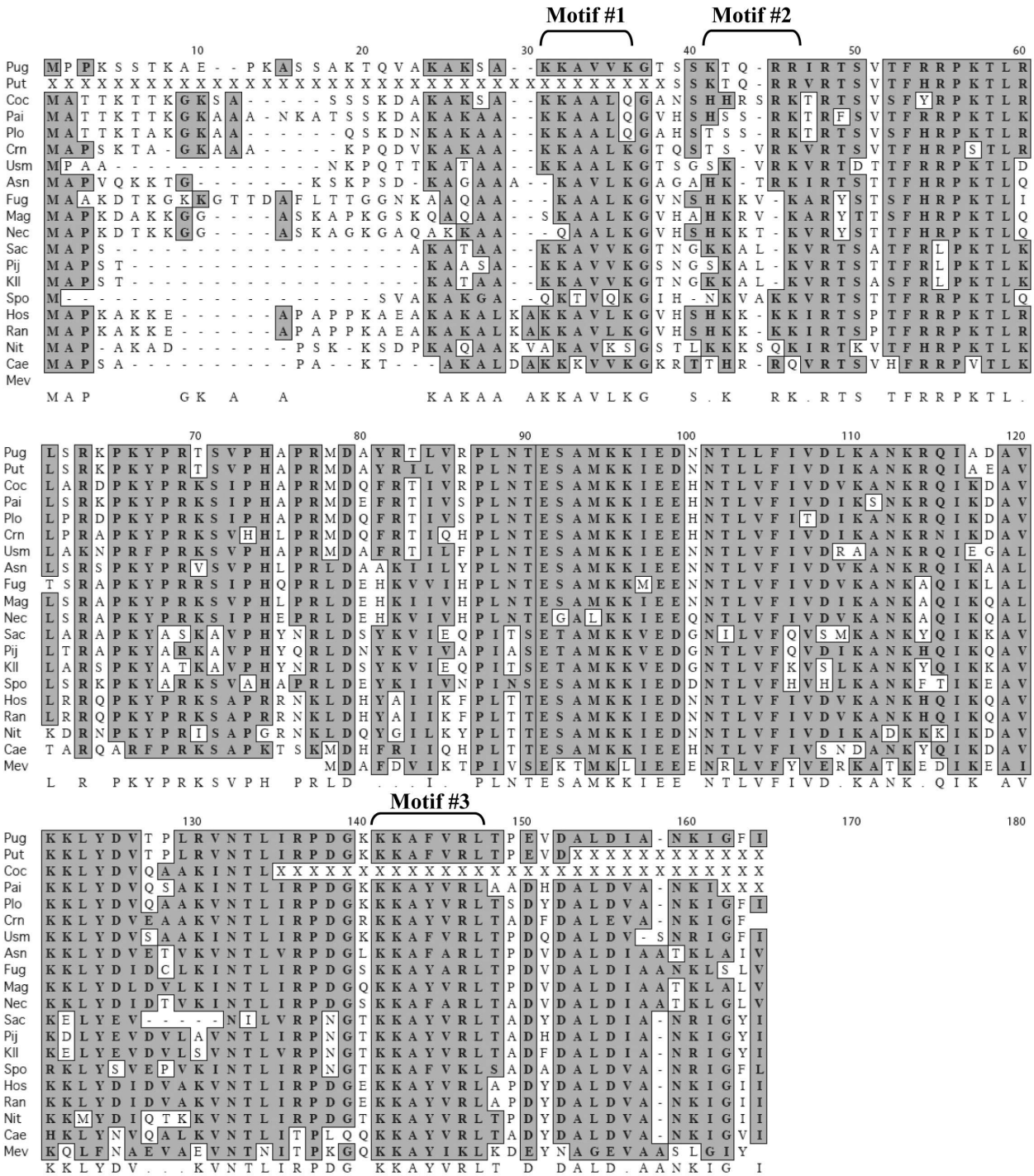


Fig. 2. Alignment of the deduced L23/L25 amino acid sequences from *P. graminis* (Pug) and 14 other fungal species and five outgroups. Codes and types of organisms are listed in Table 1. Gray shaded areas indicate consensus sequences, which includes conserved substitutions. Missing data (X) and gaps (-) are indicated.

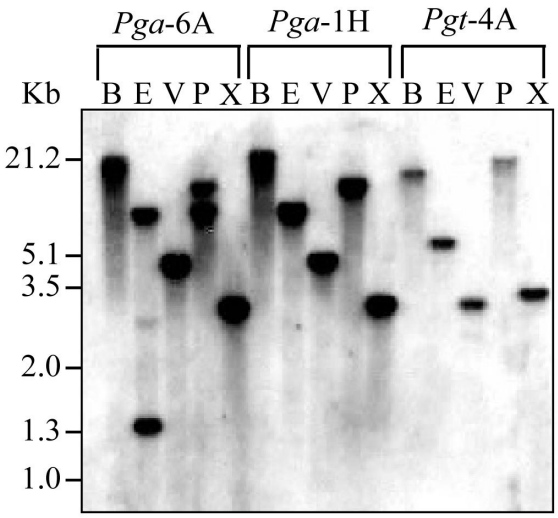


Fig. 3. DNA blot analysis of genomic DNA from *Puccinia graminis* probed with pCRL130. Molecular weight markers are shown and denoted in kilobase pairs. B, *Bam*HI; E, *Eco*RI; V, *Eco*RV; P, *Pst*I; X, *Xho*I.

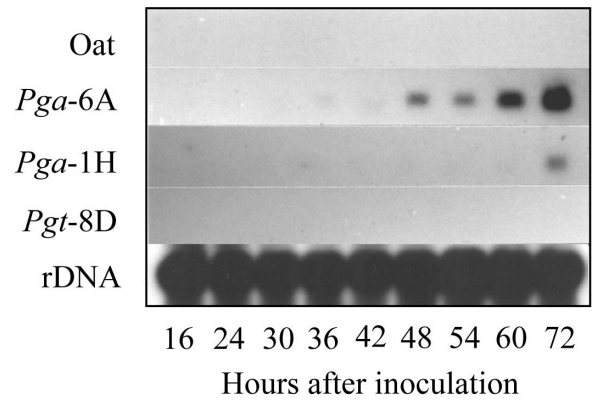


Fig. 4. Temporal accumulation patterns of the L25 transcripts in three isolates of *Puccinia graminis*. Total RNA was isolated from eight-day-old seedling leaves of oat or from oat leaves infected with compatible isolate *Pga*-6A, incompatible isolate *Pga*-1H, or inappropriate isolate *Pgt*-8D from 16 to 72 h after inoculation.

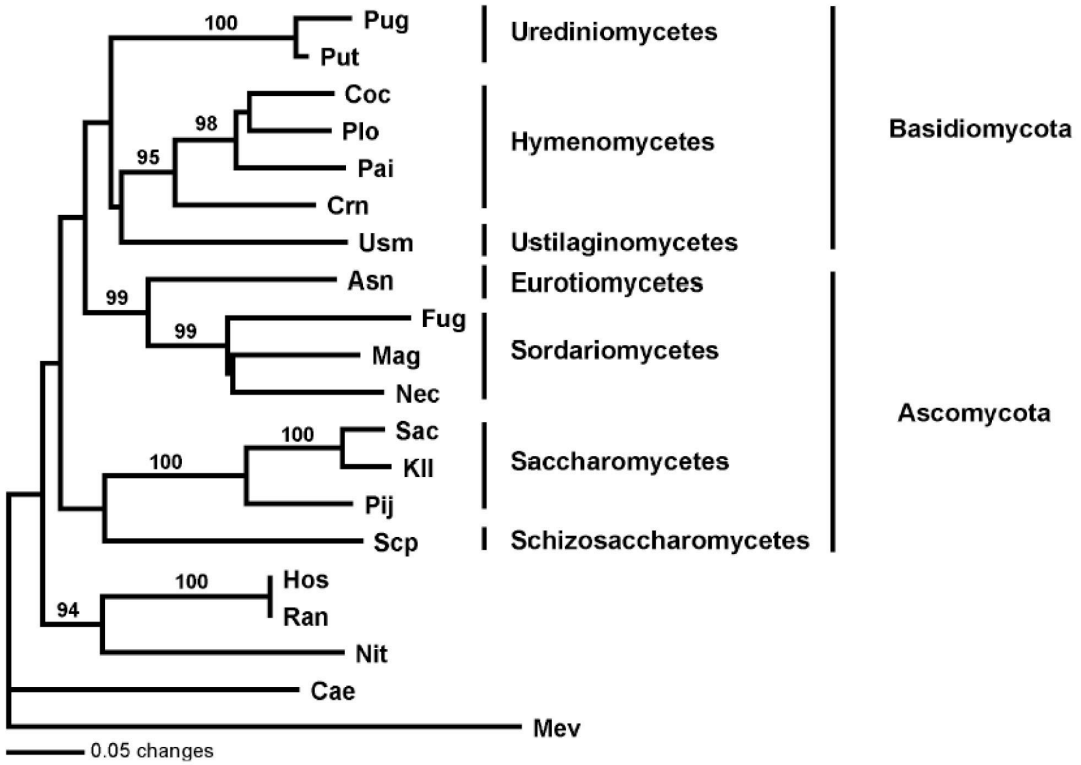


Fig. 5. Phylogenetic tree obtained from Neighbor Joining analysis of aligned amino acid sequences of *Puccinia graminis* L25 (Pug) and 14 other fungal L23/L25 ribosomal proteins. Species, code, type of organism, and source are listed in Table 1. Five additional sequences are included as outgroups. Numbers alongside branches represent percentages of congruent clusters in 1000 bootstrap trials. Branch lengths are proportional to number of amino acid changes.

branched colonies 280 μm long by 72 h AI. With the incompatible isolate (*Pga*-1H), colony development was slower and transcripts were not clearly evident until 72 h AI. At that time, *Pga*-1H colonies were only 120 μm long [12] and amounts of transcript were much smaller than that of compatible *Pga*-6A (Fig. 4). With inappropriate isolate *Pgt*-8D, colonies were very slow growing, never exceeding 85 μm in length or giving visible infection sites on leaves. With this isolate, no transcripts accumulated by 72 h AI. Likewise, no transcripts accumulated in uninoculated control tissues. These results indicated that the amount of L25 transcript expression correlated with the amount of fungal growth in each host-parasite combination. Previous observations had shown that L23/L25 and other ribosomal protein genes are expressed in amounts that parallel cell growth [32]. The *P. graminis* L25 may be useful as a marker for studying amounts of fungal development within rusted plants.

### Phylogenetic analysis

Twenty aligned L23/25 amino acid sequences containing currently available fungal sequences (Fig. 2, Table 1) and representatives of other eukaryotic and prokaryotic sequences were used for phylogenetic analysis (Fig. 5). This divided the basidiomycetes into three well-supported lineages: Urediniomycetes (rusts; *P. graminis* [Pug], *P. triticina* [Put]), Hymenomycetes (mushrooms and jelly fungi; *Coprinus cinereus* [Coc], *Paxillus involutus* [Pai], *Pleurotus ostreatus* [Plo], *Cryptococcus neoformans* [Crn]) and Ustilaginomycetes (smuts; *Ustilago maydis* [Usm]). This result is consistent with the current phylogenetic division of Basidiomycota based on ribosomal DNA sequencing [1, 23, 24] and represents the first non-ribosomal gene analysis to confirm this. In addition, the subdivision of the Hymenomycetes into Homobasidiomycetes (*Coprinus cinereus* [Coc], *Paxillus involutus* [Pai], *Pleurotus ostreatus* [Plo]) and Heterobasidiomycetes (*Cryptococcus neoformans* [Crn]) is well supported. Furthermore, the eight ascomycetes examined were divided into four well-supported lineages Eurotiomycetes (*Aspergillus nidulans* [Asn]), Sordariomycetes (*Fusarium graminearum* [Fug], *Magnaporthe grisea* [Mag], *Neurospora crassa* [Nec]), Saccharomycetes (*Kluyveromyces lactis* [Kll], *Pichia jadinii* [Pij], *Saccharomyces cerevisiae* [Sac]) and Schizosaccharomycetes (*Schizosaccharomycetes pombe* [Scp]). This is consistent with current taxonomic classification. These results indicate that the nuclear ribosomal L23/25 protein provides a useful gene for phylogenetic analysis of fungi.

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# 摘 要

林國知[1, 3], Bushnell, W. R.[2], and Szabo, L. J.[2] 2004. 桿銹菌核糖體蛋白L25 之分子特性. 植病會刊 13：251-260. ([1.] 國立東華大學生命科學系；[2.] 美國明尼蘇達大學植物病理學系暨美國農業部穀類疾病實驗室；[3.] 聯絡作者，電子郵件：kclin@mail.ndhu.edu.tw；傳眞：+886-3-8633630)

從燕麥桿銹菌 (*Puccinia graminis* f. sp. *avenae*) 分離出 25S rRNA-結合蛋白 (L25) 之 cDNA。此 cDNA 長度爲 660 個鹽基對 (bp)，可合成一條 158 個胺基酸之胜肽。L25 之胺基酸序列與其它生物之核糖體蛋白 L23/L25 有極高之相似度。當燕麥桿銹菌感染宿主時，最快可於 48 小時偵測到 L25 基因之RNA；而 L25 基因之RNA 累積的時間與桿銹菌致病株生長的時間吻合。由L23/L25 蛋白之演化遺傳分類分析顯示燕麥桿銹菌 L25 蛋白與其它眞菌之 L25 蛋白關係親近，且可區分 Urediniomycetes, Hymenomycetes 及 Ustilaginomycetes (皆屬 Basidiomycota)，此分類結果與現有之分類相同。此外，以 L23/L25 蛋白分析其對Ascomycota之演化遺傳其分類結果亦與現有之分類結果吻合。此報告爲第一次以分析非核糖體RNA 基因證實 Basidiomycota 之分類，且顯示 L23/L25 胺基酸序列可作爲研究眞菌演化遺傳分類之工具。

關鍵詞：*Puccinia graminis*、桿銹菌、核糖體蛋白L25、rRNA-結合蛋白、演化遺傳、擔子菌綱