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Genomic Structure of the Cu,Zn Superoxide Dismutase (SOD1) Gene of *Paecillomyces tenuipes* and *Paecilomyces* sp.

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We describe here the complete nucleotide sequence and the exon-intron structure of the Cu,Zn superoxide dismutase (SOD1) gene of *Paecilomyces tenuipes* and *Paecilomyces* sp. The SOD1 gene of *P. tenuipes* spans 966 bp, and consisted of three introns and four exons coding for 154 amino acid residues. Three unambiguous introns in *P. tenuipes* separate exons of 13, 332, 97, and 20 bp, all exhibiting exon sizes identical to *Cordyceps militaris* SOD1 gene. The SOD1 gene of *Paecilomyces* sp. contains 946 bp and consisted of four introns and five exons coding for 154 amino acid residues. Five exons of *Paecilomyces* sp. SOD1 are composed of 13, 180, 152, 97, and 20 bp. Interestingly, this result showed that the total length of exons 2 (180 bp) and 3 (152 bp) of *Paecilomyces* sp. SOD1 is same to exon 2 length (332 bp) of *C. militaris* SOD1 and *P. tenuipes* SOD1. The deduced amino acid sequence of the *P. tenuipes* SOD1 showed 95% identity to *C. militaris* SOD1 and 78% to *Paecilomyces* sp. SOD1. Phylogenetic analysis confirmed that the *C. militaris* SOD1, *P. tenuipes* SOD1 and *Paecilomyces* sp. SOD1 are placed together within the ascomycetes group of fungal clade.

Key words: Entomopathogenic fungi, Genomic structure, *Paecilomyces tenuipes*, *Paecilomyces* sp., Phylogenetic analysis, Superoxide dismutase (SOD1), Vegetable wasp and plant worm

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Introduction

Vegetable wasp and plant worm is an entomopathogenic fungus which attacks larva, pupa or adult of host insects. Generally, vegetable wasp and plant worm indicates the fruiting body formed on the larval, pupal or adult integument of its insect host, which is insect-born mushroom showing special appearance produced from the host insects attacked by entomopathogenic fungi (Samson *et al.*, 1988; Shimizu, 1997). In the Orient, vegetable wasp and plant worm is one of the most potent herbs in traditional medicine and used widely as a tonic for longevity, endurance and vitality. Along with an extensive medicinal interest, a great variety of vegetable wasp and plant worm have been collected and a few of them, especially *Cordyceps sinenesis*, *C. militaris* and *Paecilomyces tenuipes*, are under investigating for their pharmaceutical use. It has been reported that vegetable wasp and plant worm can produce many kinds of bioactive compounds and the medicinal benefits (Kneifel *et al.*, 1977; Furuya *et al.*, 1983; Yamada, 1984; Montefiori *et al.*, 1989; Xu *et al.*, 1992; Carlile and Watkinsom, 1996; Kuo *et al.*, 1996; Zhu *et al*, 1998a, b; Nakamura *et al.*, 1999; Yamaguchi *et al.*, 2000). An artificial in vivo mass-production technology of *C. militaris* and *P. tenuipes* was established in live silkworm, *Bombyx mori*, pupae as a host (Lee *et al.*, 2001a, b).

Superoxide dismutase (SOD), one of the essential element of the antioxidant defense system, mainly removes O_2^- and also prevents O_2^- mediated reduction of iron and subsequent OH^- generation, which is highly toxic to the organism. Of these SOD enzymes, Cu,Zn-containing SOD (SOD1) is an important component of the antioxidant defense system in eucaryotic cells (McCord and Fri-

dovich, 1969; Fridovich, 1986; Crapo *et al.*, 1992). The SOD1 enzyme binds one copper and one zinc ion and displays the Greek Key β-barrel fold (Tainer *et al.*, 1982). The SOD1 has also been identified in various species such as fungi, plants, insects, and mammals, and its gene also has been subjected to investigation in molecular and cellular level. Also, molecular characterization of SOD1 has been studied in various fungi species (Chary *et al.*, 1994; Jamieson *et al.*, 1994; Holdom *et al.*, 2000; Oberegger *et al.*, 2000; Chaturvedi *et al.*, 2001).

Previously, in *C. militaris*, the SOD1 gene has been reported in our laboratory (Park *et al.*, 2005). It is the first report of SOD1 gene in entomopathogenic fungi. Our current study focuses on genomic structure of SOD1 gene in *P. tenuipes* and *Paecilomyces* sp. Here, we present the exon/inron structure and phylogenic relationship of SOD1 gene from *P. tenuipes* and *Paecilomyces* sp.

Materials and Methods

Fungus

The entomopathogenic fungus, *Paecilomyces tenuipes* (Bae *et al.*, 2002), was artificially cultured in the silkworm (*Bombyx mori*) pupae as described previously (Lee *et al.*, 2001a, b). *Paecilomyces* sp. (Chen *et al.*, 2001) is considered as the anamorph of *C. sinensis*, and was isolated from fresh fruiting body of *C. sinensis*.

Genomic DNA isolation and PCR of the SOD1 gene

Genomic DNA was extracted from the fruiting body of *P. tenuipes* and *Paecilomyces sp.* using a Wizard™ Genomic DNA Purification Kit, according to the manufacturer's instructions (Promega, Madison, WI). The primers used for amplification of a genomic DNA encoding the SOD1 were 5'-GACAAAATCATCCAAAATGGTCAAAGCAG-3' for the translational start sequence region and 5'-GCCTCTTAGTTGGCGACGCCAATGACAC-3' for the 3' coding region, based on the sequence of *C. militaris* SOD1 cDNA cloned in our previous study (Park *et al.*, 2005). After a 35-cycle amplification (94°C for 30 sec; 50°C for 40 sec; 72°C for 2 min), PCR products were analyzed with 1.0% agarose gel electrophoresis. The PCR product was then purified with a PCR Purification Kit (Qiagen) following manufacturer's instruction and cloned into pGem-T vector (Promega).

Genomic DNA sequencing and data analysis

Sequence of each genomic DNA was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program. MacVector (ver. 6.5, Oxford Molecular Ltd) was used to align the amino acid sequences of SOD1. With the twenty-five GenBank-registered SOD1 amino acid sequences, phylogenetic analysis was performed using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0 (Swofford, 2000). The accession numbers of the sequences in the GenBank are as follows: *Paecilomyces tenuipes* (AY176060; this study), *Paecilomyces* sp. (AY438328; this study), *Cordyceps militaris* (AY176061), *Claviceps purpurea* (AJ344050), *Neurospora crassa* (M58687), *Emericella nidulans* (AF305546), *Aspergillus fumigatus* (AF128886), *Debaryomyces hansenii* (AF016383), *Candida albicans* (AF046872), *Saccharomyces cerevisiae* (P00445), *Debaryomyces vanrijiae* var. *vanrijiae* (AF301019), *Filobasidiella neoformans* var. *bacillispora* (AF248051), *Schizosaccharomyces pombe* (AL121770), *Cryptococcus neoformans* var. *neoformans* (AF248048), *Danio rerio* (Y12236), *Oncorhynchus mykiss* (AF469663), *Mus musculus* (XM128337), *Rattus norvegicus* (Y00404), *Prionace glauca* (S45643), *Equus caballus* (AB001692), *Homo sapiens* (L44135), *Gallus gallus* (U28407), *Chymomyza amoena* (S48117), *Drosophila willistoni* (L13281), *Drosophila erecta* (AF127156), *Drosophila virilis* (X13831), *Drosophila orena* (AF127155), and *Solidago canadensis* (D49485).

Results

Genomic structure of the SOD1 gene of *P. tenuipes* and *Paecilomyces* sp.

To identify the genomic structure of the SOD1 gene of *P. tenuipes* and *Paecilomyces* sp., we designed a primer set based on the sequences of the *C. militaris* SOD1 gene cloned previously in our laboratory (Park *et al.*, 2005). The genomic DNA of the SOD1 gene from the three species was cloned and sequenced.

In case of *P. tenuipes*, the nucleotide sequence also reveals that the SOD1 gene contains 966 bp and consisted of three introns and four exons coding for 154 amino acid residues (Fig. 1). The coding sequence is interrupted by three unambiguous introns with lengths of 390, 60, and 54 nucleotides. The three introns separate exons of 13, 332, 97, and 20 bp, all exhibiting exon sizes identical to *C. militaris* SOD1 gene (Park *et al.*, 2005).

On the other hand, genomic structure of *Paecilomyces* sp. SOD1 gene differed from the *P. tenuipes* and *C. militaris* (Park *et al.*, 2005) by the number of intron and exon, but was identical to the coding sequence size (154 amino acid residues) of *P. tenuipes* and *C. militaris* (Fig. 2). The

(a)

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-16                                                 gacaaaatcatccaaa
     Exon1          Intron1
  1  ATGGTCAAAGCAGgtaaggaatcaagaaaaacgcccatgaagcactgcactgtgtacaca
  1  M  V  K  A  V
 61  tctgcactgtcggcggatgatattggtgataaatttgatggaggggctggcagcactgga

121  tgtttatagcttgggcaagaaatgcgagctggtttgcgctggtttttttttagccgcgc

181  caccatgttccgttacggccacccggccttattattgatgaccttgatggattgggcttg

241  ctgcaagcaagaattgtctctcgattcgagggccttgatggcatccagccttgtcccgcc

301  ctgcctcgcctgccctcccctccagctgctcccctccagacgaaccccccaatgaaaccc
                                                  Exon2
361  cccatgtctctcaccagaacaaattctaaccaatcgctcccagTCTGTGTTCTCCGCGGC
  6                                                   C  V  L  R  G

421  GACGCCAAGGTTGGCGGCACCGTCACCTTTGAGCAGGAATCCGAGTCGGCGCCCACCACC
 11  D  A  K  V  A  G  T  V  T  F  E  Q  E  S  E  S  A  P  T  T

481  ATCACCTATGACCTGACCGGCAACGACCCCAACGCCGAGCGCGGCTTTCACATCCACACC
 31  I  T  W  D  L  T  G  N  D  P  N  A  E  R  G  F  H  I  H  T

541  TTTGGCGACAACACCAACGGCTGCACCTCGGCCGGCCCGCACTTCAACCCTCACGGCAAG
 51  F  G  D  N  T  N  G  C  T  S  A  G  P  H  F  N  P  H  G  K

601  ACGCATGGTGCGCCCACTGACGCCGCCCGTCACGTGGGCGACCTCGGCAACGTCAAGACG
 71  T  H  G  A  P  T  D  A  A  R  H  V  G  D  L  G  N  V  K  T

661  GACGCCCAGGGCAACGCCAAGGGTTCCATCCAGGACTCCCAGGTCAAGTTGATTGGCCCG
 91  D  A  Q  G  N  A  K  G  S  I  Q  D  S  Q  V  K  L  I  G  P
                    Intron2
721  CACAGCGTCATTGGCgtgcgtatcaatctagcgcgaacactcggtacccatcatgatctg
111  H  S  V  I  G
                    Exon3
781  ctaacatgcttctagCGCACTGTCGTTGTCCACGCCGGCACCGACGACCTCGGCAAGGGC
116                 R  T  V  V  V  H  A  G  T  D  D  L  G  K  G
                                                       Intron3
841  GGCAATGAAGAGTCCCTCAAGACTGGCAACGCCGGTCCCCGTCCCGCTTGCGgtaagact
131  G  N  E  E  S  L  K  T  G  N  A  G  P  R  P  A  C  G
                                                       Exon4
901  ctccacccccccaaagtgcttcgaaacaatactgatggtcttctagGTGTCATTGGCGTC
149                                                  V  I  G  V

961  GCCAACTAAgaggc
153  A  N  *
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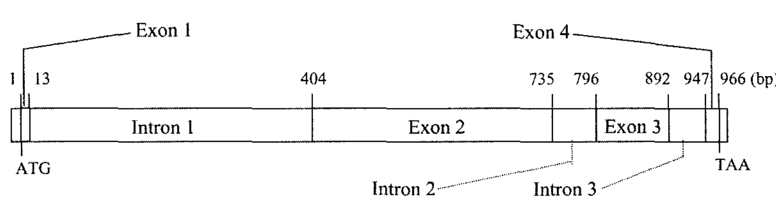
(b)



(c)

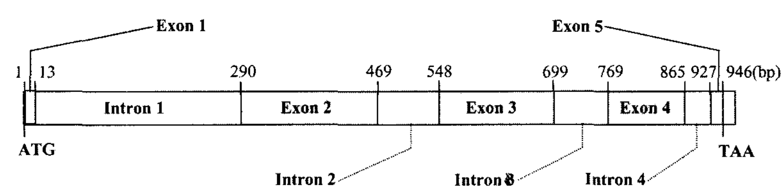
Exon	Length of exon (bp)	Position in gene	Sequence at exon-intron juntion		
1	13	1-13	M V tccactATGGTC	....	K A AAAGCAGgtaagaa
2	332	404-735	V C V gctcccagTCTGTGT	....	V I G GTCATTGGCgtgcgtat
3	97	796-892	R T V cttctagCGCACTGTC	....	A C CGCTTGCGgtaagact
4	20	947-966	G V I tcttctagGTGTCAT	....	G V A N GGCGTCGCCAACtaa

Fig. 1. The nucleotide sequence and genomic organization of *P. tenuipes* SOD1 gene. (a) Nucleotide sequence of *P. tenuipes* SOD1 gene. Nucleotide numbers are presented on the left, and the first base of initiation codon of the ORF is defined as +1. The amino acid sequence (cDNA) is shown with lower case letters. The start codon of ATG is boxed and the termination codon is shown by asterisk. Exons and introns are labeled with bold-lines. This genomic sequence has been deposited in GenBank under accession number AY176060. (b) Exon/intron structures. Numbers indicate the length (bp) of exons and introns. (c) Lengths of exons and exon/intron boundaries.

(a)

```
-16                                                         gacaaaatcatccaaa
     Exon1        Intron1
  1  ATGGTCAAAGCAGgtatgggaaccacgtgcgcccctcccgtcaaagtctcggctgcgtcc
  1  M  V  K  A  V
 61  gaagggggcggctctctttgtcagtgcgagggggttggcgctcatttgagcgagtttctg
121  tcgtatccgccgacacctcgttggacaatcggatcgcacgcaaccttgatggctgccagc
181  cctatccgcaatcagatgaagcctggacagagtcccttgatgggcagcatatcgttttcg
                                                          Exon2
241  tcgtctgcataagtatggcgtcgaggctaatccgtcgtgtgcgtgacagTTTGCGTCCTT
  6                                                    C  V  L
301  CGCGGCGACTCCAAGATCACCGGGATCGTCAATTTTGAGCAGGAGTCCGATTCCTCGCCC
  9  R  G  D  S  K  I  T  G  I  V  N  F  D  Q  E  S  D  S  S  P
361  ACCACCATCTCCTGGGAGATCTCTAACCACGATGCCGACGCCAAGCGTGGCTTCCACATC
 29  T  T  I  S  W  E  I  S  N  H  D  A  D  A  K  R  G  F  H  I
                                                        Intron2
421  ACACCCTTTGGTGACAATACCAACGGCTGCACCTCTGCTGGCCCGCACTgtaagacgtcc
 49  T  P  F  G  D  N  T  N  G  C  T  S  A  G  P  H  F
481  aatgaggttgtccgtatctcttgataagctcagagcagttctgctgacacttgtctacct
           Exon3
541  cctgcagTTAACCCTCACGGCAAGACTCACGGCAACGTGACCGACGAAAACCGACACGTT
 66           N  P  H  G  K  T  H  G  N  V  T  D  E  N  R  H  V
601  GGCGACATGGGCAACATCGAGACCGATTGCGACGGCAACTCCAAGGGATCCATAAAGGAC
 83  G  D  M  G  N  I  E  T  D  C  D  G  N  S  K  G  S  I  K  D
                                                  Intron3
661  AAGCTCATCAAGCTCATTGGCCCCCACAGTGTCATTGGCgtacgtcgagcattccctgca
103  K  L  I  K  L  I  G  P  H  S  V  I  G
                                                        Exon4
721  gcaaattattaggagacgatagttggcaagagtactcacttctgatagCGCACCGTTGTC
116                                                    R  T  V  V
781  ATTCACGCCGGCACTGATGACCTAGGCAAGGGTGGCAACGACGAGTCACTCAAGACCGGC
120  I  H  A  A  T  D  D  L  G  K  G  G  N  D  E  S  L  K  T  G
                              Intron4
841  AATGCTGGCCCCCGTCCTGCTTGCGgttcgcgtcccctaaattggtttctgcactcaaga
140  N  A  G  P  R  P  A  C  G
                                    Exon5
901  tcgtctctgctaacgagttgaactagGTGTCATTGGCGTCGCCAACTAAgaggc
149                                 V  I  G  V  A  N  *
```

(b)



(c)

Exon	Length of exon (bp)	Position in gene	Sequence at exon-intron juntion
1	13	1-13	M C ...... K A atccaaATGGTC ..... AAAGCAGgtatgggaa
2	180	290-469	V C V ...... G P H cgtgacagTTTGCGTC ..... GCCCGCACTgtaagacg
3	152	548-699	F N P ...... V I G cctgcagTTAACCCT ..... GTCATTGGCgtacgtcg
4	97	769-865	A T V ...... A C ctgatagCGCACCGTT ..... TGCTTGCGgttcgcgtc
5	20	927-946	G V I ...... G V A A ttgaactagGTGTCAT ..... GGCGTCGCCAACtaa

Fig. 2. The nucleotide sequence and genomic organization of *Paecilomyces sp.* SOD1 gene. (a) Nucleotide sequence of *Paecilomyces sp.* SOD1 gene. Nucleotide numbers are presented on the left, and the first base of initiation codon of the ORF is defined as +1. The amino acid sequence (cDNA) is shown with lower case letters. The start codon of ATG is boxed and the termination codon is shown by asterisk. Exons and introns are labeled with bold-lines. This genomic sequence has been deposited in GenBank under accession number AY438328. (b) Exon/intron structures. Numbers indicate the length (bp) of exons and introns. (c) Lengths of exons and exon/intron boundaries.

coding sequence of *Paecilomyces* sp. is interrupted by four unambiguous introns with lengths of 276, 78, 69, and 61 nucleotides, and the four introns separate exons of 13, 180, 152, 97, and 20 bp.

The SOD1 coding sequence with 154 amino acid residues among three species, *P. tenuipes*, *C. militaris* and *Paecilomyces* sp., was identical to each other. As shown in the result of intron boundaries, the consensus sequences, including an invariant GT at the intron 5' boundary and an invariant AG at its 3' boundary were very well conserved in two species, as is true for fungal SOD1 genes, *N. crassa* (Chary *et al.*, 1990), *C. neoformans* (Chaturvedi *et al.*, 2001), *C. purpurea* (Moore *et al.*, 2002), and *C. militaris* (Park *et al.*, 2005).

Comparison of the amino acid sequences of the SOD1 gene of *P. tenuipes* and *Paecilomyces* sp.

Pairwise comparison among amino acid sequences of the *C. militaris* SOD1, *P. tenuipes* SOD1 and *Paecilomyces* sp. SOD1 revealed high homology among them. The *C. militaris* SOD1 showed 95% identity to the *P. tenuipes* SOD1 and 78% identity to the *Paecilomyces* sp. SOD1 (Table 1).

Further, comparison of *C. militaris* SOD1 with *P. tenuipes* SOD1 and *Paecilomyces* sp. SOD1 revealed the presence of six His and one Asp residues (Fig. 3), which are known to act as the metal binding ligands in other fungal SOD1 sequences (Chary *et al.*, 1990; Holdom *et al.*, 2000; Chaturvedi *et al.*, 2001). The metal binding sites, His47,

Table 1. Pair-wise comparisons among amino acid sequences of SOD1 genes obtained from this study and known SOD1 genes obtained through GenBank search

	Percent Similarity													
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *C.militaris*		98	92	87	90	83	84	83	84	85	79	81	77	78
2. *P. tenuipes*	95		92	89	91	85	85	85	84	86	79	82	79	79
3. *C. purpurea*	88	87		89	90	83	83	83	83	83	79	78	77	79
4. *N. crassa*	82	82	85		88	82	82	79	83	83	75	77	79	78
5. *Paecilomyces* sp.	78	79	79	76		82	84	86	82	82	80	79	78	80
6. *E. nidulans*	75	76	75	74	71		93	83	84	81	77	76	80	75
7. *A. fumigatus*	75	75	74	74	73	90		83	84	78	78	78	79	80
8. *D. hansenii*	74	75	75	70	72	72	73		87	83	93	76	74	76
9. *C. albicans*	74	73	72	74	68	75	75	77		80	83	78	72	76
10. *S. cerevisiae*	74	74	74	70	70	72	67	72	68		77	76	76	74
11. *Dv* var. *vanrijiae*	69	69	69	65	66	65	67	92	72	66		73	70	72
12. *Fn* var. *bacillispora*	68	70	68	66	65	65	69	67	66	64	63		78	86
13. *S. pombe*	65	68	68	69	64	71	68	63	61	62	58	64		78
14. *Cn* var.*neoformans*	64	66	65	64	64	63	67	64	63	62	60	79	64	
15. *D. rerio*	62	64	62	61	59	57	59	61	56	59	60	63	57	64
16. *O. mykiss*	61	62	58	59	59	55	57	57	54	56	55	61	55	61
17. *M. musculus*	58	59	57	55	56	56	58	56	56	54	54	63	53	66
18. *R. norvegicus*	57	59	56	53	55	56	57	55	55	55	53	64	54	66
19. *P. glauca*	57	59	58	55	55	56	59	55	55	55	52	62	53	64
20. *E. caballus*	57	57	56	53	53	53	56	56	55	56	54	63	54	65
21. *H. sapiens*	57	58	55	54	56	55	59	57	54	54	55	63	55	65
22. *G. gallus*	57	59	55	52	52	53	55	55	53	53	53	63	54	65
23. *C. amoena*	56	55	55	53	53	53	54	53	56	51	52	55	52	56
24. *D. willistoni*	56	57	55	55	53	55	55	55	55	50	54	57	53	57
25. *D. erecta*	56	57	55	55	57	55	56	54	57	51	53	57	53	57
26. *D. virilis*	55	56	55	53	53	54	55	54	55	50	53	57	51	58
27. *D. orena*	55	56	55	54	56	55	57	54	57	51	53	56	53	57
28. *S. canadensis*	56	60	57	57	52	55	55	56	57	55	52	58	50	57
	Percent Identity													

Table 1. Continued

Species	Percent Similarity 15	16	17	18	19	20	21	22	23	24	25	26	27	28
1. *C.militaris*	76	76	72	71	73	69	69	71	71	68	70	69	69	73
2. *P. tenuipes*	76	76	71	71	73	69	69	71	71	69	71	70	69	74
3. *C. purpurea*	71	72	70	69	74	68	66	68	71	69	71	70	70	72
4. *N. crassa*	73	74	71	69	71	66	66	67	69	68	71	68	69	71
5. *Paecilomyces* sp.	75	76	74	74	72	69	70	70	69	67	70	68	68	73
6. *E. nidulans*	68	70	70	69	70	65	67	66	67	67	68	68	68	70
7. *A. fumigatus*	69	71	72	70	73	68	71	69	68	67	68	68	69	70
8. *D. hansenii*	72	70	74	75	73	70	71	71	69	69	69	68	69	72
9. *C. albicans*	69	70	73	73	71	72	71	72	71	70	73	71	73	70
10. *S. cerevisiae*	73	72	72	73	72	70	67	68	66	65	65	68	65	68
11. *Dv* var. *vanrijiae*	70	67	72	72	69	68	69	67	68	68	67	67	67	68
12. *Fn* var. *bacillispora*	74	75	74	74	71	73	72	75	69	70	71	71	69	69
13. *S. pombe*	69	69	67	67	66	68	67	66	66	66	65	65	65	62
14. *Cn* var.*neoformans*	67	75	77	77	76	73	73	75	72	72	73	74	73	69
15. *D. rerio*		88	81	80	81	77	77	77	76	74	72	75	72	71
16. *O. mykiss*	81		82	81	79	75	78	78	75	72	75	76	75	72
17. *M. musculus*	71	71		98	80	85	88	82	75	74	73	76	73	69
18. *R. norvegicus*	70	69	96		79	86	90	84	75	73	73	75	73	67
19. *P. glauca*	74	69	73	71		78	75	73	71	71	71	73	71	71
20. *E. caballus*	67	64	81	81	69		87	81	70	71	68	72	69	64
21 *H. sapiens*	70	68	83	83	68	80		83	74	72	72	71	72	63
22. *G. gallus*	66	62	70	70	64	72	71		70	71	70	72	71	64
23. *C. amoena*	62	61	59	58	60	57	59	58		91	90	90	91	74
24. *D. willistoni*	62	60	60	59	61	59	60	60	86		89	92	90	76
25. *D. erecta*	59	62	59	59	58	56	61	58	83	86		89	98	75
26. *D. virilis*	62	62	60	59	60	58	59	60	85	90	84		90	77
27. *D. orena*	59	62	59	59	58	57	61	59	83	86	98	85		75
28. *S. canadensis*	57	58	57	55	58	53	53	53	58	62	60	62	60	

Percent Identity

49, 64, 121 for copper and His64, 72, 81 and Asp84 for zinc, were conserved in all SOD1 sequences from three species (Fig. 3). The residue Arg144 considered as important residue for SOD1 enzyme activity (Malinowski and Fridovich, 1979; Borders *et al.*, 1985) and two cysteine residues at positions 58 and 147 for intrachain disulfide bridge are well conserved in three species.

Phylogenetic analysis using the SOD1 gene

A phylogenetic analysis using the deduced amino acid sequences of known SOD1 genes derived from fungi, vertebrates, insects and plants revealed that the SOD1 is divided into four separate clades (Fig. 4). Within the fungal clades, the SOD1 from ascomycetes (*C. militaris, C. purpurea, N. crassa, E. nidulans, A. fumigatus, D. hansenii, Dv* var. *vanrijiae, C. albicans, S. cerevisiae* and *S. pombe*) and basidiomycetes (*Fn* var. *bacillispora* and *Cn* var. *neoformans*) formed a monophyletic group, respectively. The two species of study fungi, *C. militaris* and *P. tenuipes* were placed as a sister group to *C. purpurea* within the ascomycetes SOD1 clade. On the other hand, *Paecilomyces* sp. SOD1 was placed within the ascomycetes group but the internal relationship within this group was poorly resolved.

Discussion

We describe the genomic structure and phylogenetic relationship of SOD1 gene from *P. tenuipes* and *Paecilomyces*

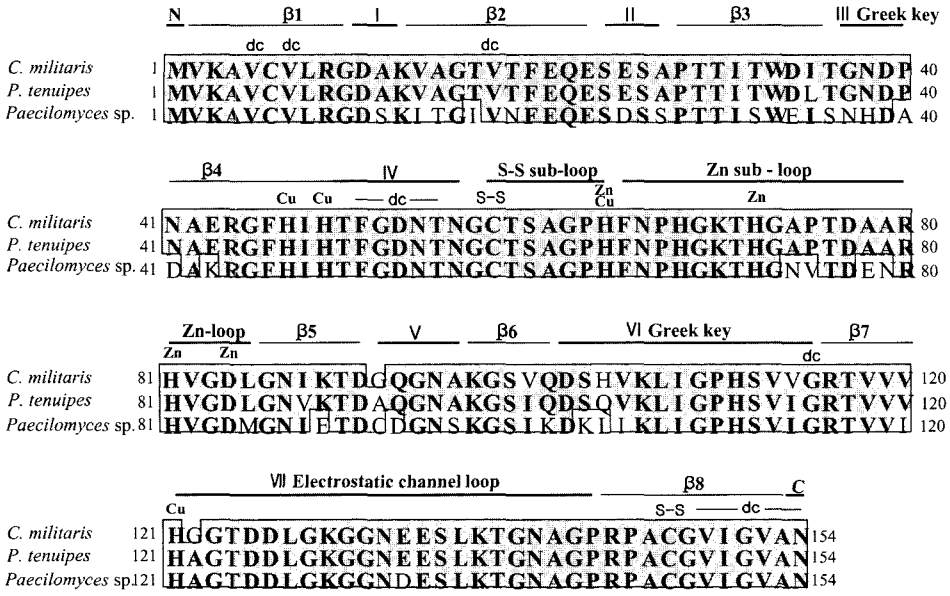


Fig. 3. Comparison of the deduced amino acid sequences of *P. tenuipes* SOD1, *C. militaris* SOD1, and *Paecilomyces* sp. SOD1. Residues are numbered according to the aligned fungal SOD1 sequences (Park *et al.*, 2005), and invariant residues are shaded black. The eight β-strands of the β-barrel, the seven connecting loops or turns (Roman numerals), and the N-terminal (N) and C-terminal (C) sequences not involved in β-strands, are shown above the alignment. Structural alignments are taken from the bovine SOD1 crystal structure (Tainer et al., 1982). Resiues that form disulfide bridge (S-S), ligate the metals (Cu or Zn), or are involved in dimmer contact (dc) are also shown above the alignment. The information of SOD1 sequences was taken from the previous fungal SOD1 studies (Steinman, 1980; Chary *et al.*, 1990; Chaturvedi *et al.*, 2001; Park *et al.*, 2005).

sp. The SOD1 genomic DNAs from *P. tenuipes* and *Paecilomyces* sp. were cloned by a PCR primer set designed based on the sequences of the C. militaris SOD1 gene cloned in our previous study (Park *et al.*, 2005). The *C. militaris* SOD1 (Park *et al.*, 2005) and *P. tenuipes* SOD1 genomic DNAs consisted of three introns and four exons coding for 154 amino acid residues. The coding sequence is interrupted by three unambiguous introns and the first intron was the largest, 335 nucleotides for C. militaris SOD1 and 390 nucleotides for *P. tenuipes* SOD1. In C. militaris and *P. tenuipes*, the three introns separate exons of 13, 332, 97, and 20 bp, all exhibiting identical exon sizes to each other. On the other hand, genomic structure of *Paecilomyces* sp. SOD1 revealed that the four introns separate five exons of 13, 180, 152, 97, and 20 bp. Interestingly, this result showed that each exon length of *Paecilomyces* sp. SOD1 except for exons 2 and 3 is identical to *C. militaris* SOD1 and *P. tenuipes* SOD1, and the total length of exons 2 (180 bp) and 3 (152 bp) of *Paecilomyces* sp. SOD1 is same to exon 2 length (332 bp) of *C. militaris* SOD1 and *P. tenuipes* SOD1. For *N. crassa* it has been reported that SOD1 gene has three introns and four exons encoding 154 amino acid residues (Chary *et al.*, 1990). In terms of exon length of SOD1 gene, *N. crassa* SOD1 had four exons of 13, 180, 152, and 117 bp. Interestingly, each length of exons 1 to 3 of *Paecilomyces* sp. SOD1 was identical to *N. crassa* SOD1, and the total length of exons 4 (97 bp) and 5 (20 bp) of *Paecilomyces* sp. SOD1 was same to last exon (117 bp) of *N. crassa* SOD1.

On the other hand, genomic SOD1 in three varieties of *C. neoformans* revealed five introns and six exons encoding 154 amino acid residues (Chaturvedi *et al.*, 2001). Also, the genomic SOD1 cloned from *C. purpurea* composed of five introns and six exons encoding 154 amino acid residues (Moore *et al.*, 2002). Although length of exons was different, the first and last exons of these fungal SOD1 genes were often too small (Chary *et al.*, 1990; Chaturvedi *et al.*, 2001; Moore *et al.*, 2002). In three varieties of *C. neoformans*, exons 1 and 6 of SOD1 gene composed of 3 and 4 amino acids, respectively (Chaturvedi *et al.*, 2001). The SOD1 genes of *C. militaris* and *P. tenuipes* also revealed that the first (4 amino acids) and last (6 amino acids) exons are small in size.

In *P. tenuipes* and *Paecilomyces* sp. SOD1 sequences, the conserved residues are present at the corresponding positions in SODs from the other fungal species (Chary *et al.*, 1990; Bordo *et al.*, 1994; Holdom *et al.*, 2000; Chaturvedi *et al.*, 2001; Park *et al.*, 2005), representing regions involved in metal binding and catalysis. Espe-

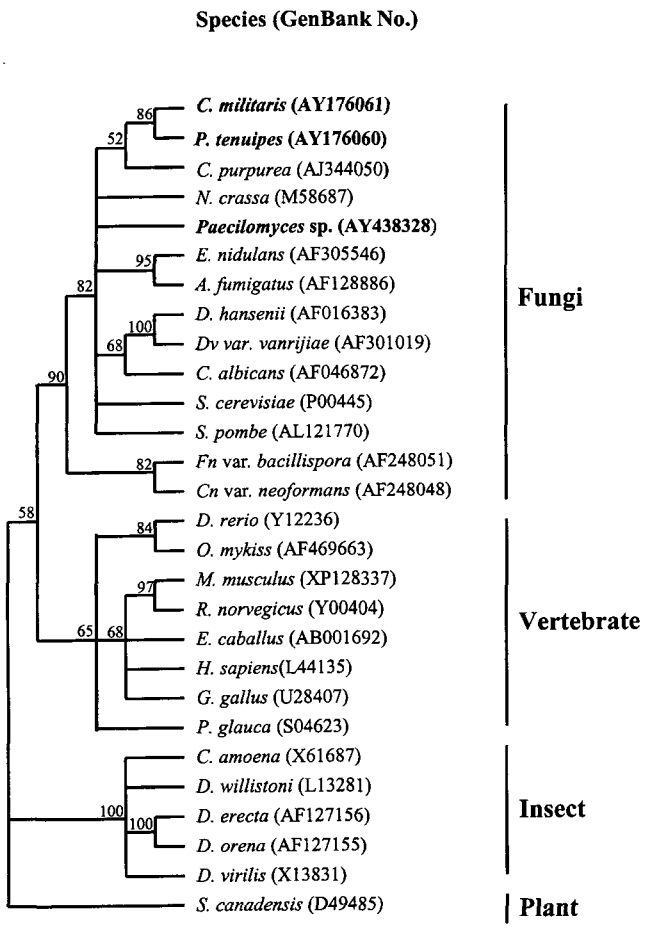


Fig. 4. Phylogenetic relationship among SOD1 sequences. GenBank accession numbers of the SOD1 sequences used in the comparison are described in Materials and Methods. The tree was obtained by bootstrap analysis with the option of heuristic search and the numbers on the branches represent bootstrap values for 1,000 replicates. The outgroup chosen was *S. canadensis* on the basis of sequence homology by pairwise comparison.

cially, copper binding sites (His47, 49, 64, and 121) and zinc binding sites (His64, 72, 81, and Asp84) are well conserved among three species.

Comparison of SOD1 sequence among three species revealed that the *C. militaris* SOD1 (Park *et al.*, 2005) showed 95% identity with the *P. tenuipes* SOD1 and 78% identity with the *Paecilomyces* sp. SOD1. Considering that SOD1 sequences from three species are highly similar to each other and, in particular, SOD1 sequences of N-termini including β1 strand and C-terminal including β8 strand are completely identical in three species, the PCR primer set designed based on the sequences of the *C. militaris* SOD1 gene in our previous study (Park *et al.*, 2005) is reasonable to serve as the primer for the amplification of genomic SOD1 DNA from three species.

A phylogenetic tree of SOD1 protein sequences placed fungi into one inclusive group, excluding other organism. Interestingly, the fungal SOD1 genes were further separated into two monophyletic groups, ascomycetes and basidiomycetes. Reasonably, the three studied species were well grouped together with other members of ascomycetes. However, an exact position within ascomycetes group was not resolved, possibly due to limited difference among the species of ascomycetes. Thus, further research on these species are required.

Currently, the promoter regions of SOD1 genomic DNA from two species are not included in this study. We are now interested in the exon-intron structure of SOD1 gene of *P. tenuipes* and *Paecilomyces* sp. More detailed analysis on the structure and regulation for these SOD1 genes will be required to understand the role of SOD1 in the entomopathogenic fungi.

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