

Comparison of genetic structure of the Cu,Zn superoxide dismutase (SOD1) from *Cordyceps militaris*, *Paecilomyces tenuipes* and *P. sinensis*

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Objectives

Superoxide dismutase (SOD), one of the essential element of the antioxidant defense system, mainly removes O₂⁻ and also prevents O₂⁻ 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. The SOD1 enzyme binds one copper and one zinc ion and displays the Greek Key β-barrel fold. The SOD1 has 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.

Previously, the SOD1 cDNA of *C. militaris* has been reported in our laboratory as the first report of SOD1 gene in the entomopathogenic fungi. Our current study is focused on genomic structure of SOD1 gene in *C. militaris*, *P. tenuipes* and *P. sinensis*. Here, we present the result of the complete nucleotide sequence and the exon-intron structure of SOD1 gene from *C. militaris*, *P. tenuipes* and *P. sinensis*.

Materials and Methods

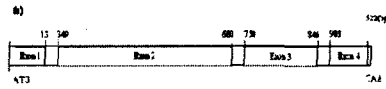
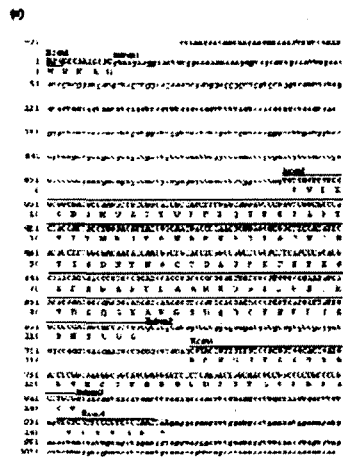
Materials - The entomopathogenic fungi, *Cordyceps militaris* and *Paecilomyces tenuipes*, and *P. sinensis* considered as the anamorph of *C. sinensis*.

Methods - Genomic DNA isolation and PCR of the SOD1 gene, Genomic DNA sequencing and data analysis

Results and Discussion

We describe here the complete nucleotide sequence and the exon-intron structure of the Cu,Zn superoxide dismutase (SOD1) gene of *Cordyceps militaris*, *Paecilomyces tenuipes* and *P. sinensis*. The SOD1 gene of *C. militaris* and *P. tenuipes* spans 922 bp and 966 bp, respectively, and consisted of both four exons coding for 154 amino acid residues and three interspersed introns, and each exon length is identical (Fig. 1 and 2). On the other hand, the SOD1 gene of *P. sinensis*, which contains 946 bp is consisted of five exons coding for 154 amino acid residues and four interspersed introns (Fig. 3). Interestingly, the total length of exons 2 (180 bp) and 3 (152 bp) of *P. sinensis* SOD1 is same to that of exon 2 (332 bp) of *C.*

militaris SOD1 and *P. tenuipes* SOD1 (Fig. 3). The deduced amino acid sequence of the *C. militaris* SOD1 showed 95% identity to *P. tenuipes* SOD1 and 78% to *P. sinensis* SOD1. The typical metal binding ligands of six histidines and one aspartic acid common to fungi SOD1 were all well conserved in the SOD1 of the three species (Fig. 4). Phylogenetic analysis placed the *C. militaris* SOD1 and *P. tenuipes* SOD1 in a relatively strong cluster (86% bootstrap value), and *P. sinensis* SOD1 were unresolved within the ascomycetes group of fungal clade (Fig. 5).

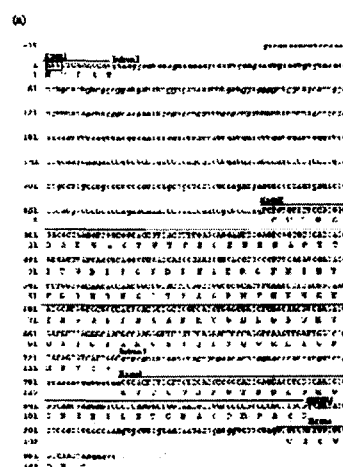


(c)

Exon	Length/Intron (bp)	Position (bp)	Sequence of exon/intron boundary
1	13	13	ATAAATGTCGTC ... K A A ATAAAGAGAGAG
2	323	240-488	GTCTGCTCTGCT ... G T A A GTCTGCTCTGCT
3	81	178-266	CTTCTGCTCTGCT ... A C G CTTCTGCTCTGCT
4	20	610-632	ATGCTCTGCT ... G T A A ATGCTCTGCT

Fig. 1. The nucleotide sequence and genomic organization of *C. militaris* SOD1 gene. (a) Nucleotide sequence of *C. militaris* 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 AY176061. (b) Exon/intron structures. Numbers indicate the length (bp) of exons and introns. (c) Lengths of exons and exon/intron boundaries.



(c)

Exon	Length/Intron (bp)	Position (bp)	Sequence of exon/intron boundary
1	13	143	ATAAATGTCGTC ... K A A ATAAAGAGAGAG
2	323	46-723	GTCTGCTCTGCT ... G T A A GTCTGCTCTGCT
3	81	178-266	CTTCTGCTCTGCT ... A C G CTTCTGCTCTGCT
4	20	610-632	ATGCTCTGCT ... G T A A ATGCTCTGCT

Fig. 2. 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.

Claviceps purpurea (AJ344050), *Neurospora crassa* (M58687), *Emericella nidulans* (AF305546), *Aspergillus fumigatus* (AF128886), *Debaryomyces hansenii* (AF016383), *Candida albicans* (AF046872), *Saccharomyces cerevisiae* (P00445), *Debaryomyces vanriijiae* var. *vanriijiae* (AF301019), *Filobasidiella neoformans* var. *bacillisporea* (AF248051), *Schizosaccharomyces pombe* (AL121770), and *Cryptococcus neoformans* var. *neoformans* (AF248048). 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. (b) Pairwise identities and similarities of the deduced amino acid sequence of *C. militaris* SOD1, *P. tenuipes* SOD1 and *P. sinensis* SOD1 among fungal SOD1 sequences.

References

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