

cDNA Cloning, Expression, Purification, and Enzyme Assay of a Cu,Zn Superoxide Dismutase (SOD1) of *Cordyceps militaris*

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A cDNA encoding the Cu,Zn superoxide dismutase (SOD1) of *Cordyceps militaris*, which is one of the entomopathogenic fungi called a vegetable wasp and plant worm, was cloned and characterized. The SOD1 cDNA contains an open reading frame of 462 bp encoding 154 amino acid residues. The deduced amino acid sequence of the *C. militaris* SOD1 cDNA showed 88% identity to *Claviceps purpurea* SOD1, 82% to *Neurospora crassa* SOD1, and 75% - 64% to other fungi SOD1. The *C. militaris* SOD1 possesses the typical metal binding ligands of six histidines and one aspartic acid common to fungi SOD1. Phylogenetic analysis confirmed a closer relationship of the deduced amino acid sequences of the *C. militaris* SOD1 gene to the *C. purpurea* and *N. crassa* within the ascomycetes group. The cDNA encoding *C. militaris* SOD1 was expressed as a 17-kDa polypeptide in the baculovirus-infected insect Sf9 cells and the enzyme activity of the purified recombinant *C. militaris* SOD1 was approximately 568 U per mg of recombinant SOD1. Southern blot analysis of the genomic DNA suggested the presence of the *C. militaris* SOD1 gene as a single copy and Northern blot analysis showed increment of SOD1 transcript signal with growth stage. Similarly, the *C. militaris* SOD1 enzyme assay also exhibited increment of activity with growth stage. This result of *C. militaris* SOD1 is the first molecular characterization of SOD1 gene from any entomopathogenic fungus.