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CLONING AND CHARACTERIZATION OF A COPPER/ZINC-SUPEROXIDE DISMUTASE GENE FROM *ASPERGILLUS JAPONICUS*.[1]

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ABSTRACT: In order to clone a Cu/Zn-SOD gene from *Aspergillus japonicus*, the highly conserved regions of the SOD sequence of maize and other species were compared and two oligonucleotides were synthesized. The *Aspergillus japonicus* cDNA was then used as the template to get a 0.3 kb fragment by PCR technique. The 0.3 kb DNA fragment has high homology compared with maize SOD-4 cDNA. Then we used the 0.3 kb fragment as probe to screen *Aspergillus japonicus* genomic libraries. A 0.4 kb DNA fragment from purified positive plaque was subcloned into pGEM-7zf(+) vector and sequenced. Nucleotide sequence analysis of this clone revealed that it comprised an open reading frame coding for 120 amino acid residues. The encoded polypeptide is highly homologous with other plant SODs. The residues required for coordinating copper and zinc are also conserved as they are among all reported Cu/Zn-SOD sequences.

KEYWORDS: *Aspergillus japonicus*, copper/zinc-superoxide dismutase (Cu/Zn-SOD).

INTRODUCTION

From 1939 to 1941, a copper-containing blue-green protein was isolated later proved to be important in the body's defense against free radicals. This protein, now known as superoxide dismutase (SOD), isolated from bovine erythrocyte, and later found to be in almost all living cells, was proved by McCard and Fridovich in 1969 to be an enzyme catalyzing the dismutation reaction of the superoxide anion radical (McCard and Fridovich, 1969).

O_2^-, H_2O_2, OH · , singlet oxygen, and lipid hydroperoxide are the five most important free radicals related to health and medicine. Normally the body possesses adequate defense systems against attack by oxygen free radicals. There are occasions when the defense systems become inadequate: 1. When there is excessive production (air, food, smoking, etc.). 2. When there is inadequate intake of antioxidant nutrients. 3. When receive medical treatment such as drugs, chemotherapy, and radiation therapy, etc. 4. The aging process causes reduced production of antioxidant enzymes (Su, 1993)

(1) The sequence is available from the Genome Sequence DataBase under the accession number L32834.

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