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Purification and Characterization of Iron-Containing Superoxide Dismutase from *Lentinus edodes*

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Abstract Superoxide dismutase (SOD) was purified to homogeneity from fruiting bodies of edible mushroom, *Lentinus edodes*, by ammonium sulfate precipitation, diethylaminoethyl (DEAE)-Sepharose FF ion-exchange chromatography, Sephacryl S-200 gel filtration chromatography, and preparative PAGE. The molecular weight of the purified enzyme was estimated to be approximately 54 kDa by gel filtration chromatography, and the enzyme was shown to be consisted of two identical subunits of molecular weight 27 kDa by SDS-PAGE. The isoelectric point of the enzyme was 4.9 as determined by isoelectric focusing. The enzyme had optimal pH and temperature of pH 8.0 and 20°C, respectively. The activity of the enzyme was inhibited by hydrogen peroxide, but inhibited less by cyanide and azide. The native enzyme was found to contain 0.89 g-atom of iron, 0.75 g-atom of zinc, and 0.46 g-atom of copper per mol of enzyme. Analysis of amino acids composition revealed that the SOD from *L. edodes* contained a relatively large amount of glutamic acid/glutamine, proline, cysteine, isoleucine, and leucine, but only a small amount of aspartic acid/asparagine, tyrosine, and tryptophan when compared to the other iron-containing SODs.

Key words: Superoxide dismutase, *Lentinus edodes*, purification

Superoxide dismutase (SOD; superoxide:superoxide oxidoreductase, EC 1.15.1.1) is a metalloenzyme catalyzing the dismutation of superoxide radicals (O_2^-), the product of univalent reduction of molecular oxygen, to molecular oxygen and hydrogen peroxide [11]. The highly reactive oxygen intermediates such as superoxide radicals and hydroxy radicals (OH ·) cause lipid peroxidation, denaturation of proteins, and structural damage of DNA. Therefore, SOD plays an important role in enzymatic protection against oxygen toxicity in oxygen-metabolizing organisms [12]. Besides aerobic organisms, the relationship between SOD activity and oxygen sensitivity in strict anaerobes such as *Bifidobacterium* spp. which is beneficial to the health of humans and the other animals has been reported [8, 37]. On the basis of metal cofactors in the active site of the enzymes, the enzyme has been classified as MnSODs, FeSODs, and CuZnSODs. MnSODs have been found in mitochondria and some bacteria [3, 33]. FeSODs is found in prokaryotes, primitive eukaryotes, and some plants [1, 31]. CuZnSODs is usually found not only in a wide range of eukaryotes including fungi, but also in some plant and prokaryotes [9, 18, 24]. It is known that the MnSOD and FeSOD enzymes are structurally similar, but CuZnSOD is structurally unrelated to them [5].

Until now, many studies have reported the characterization and physiological significance of SOD in fungi. For example, SODs of *Neurospora crassa*, *Saccharomyces cerevisiae*, *Candida albicans*, and *Aspergillus fumigatus* have been purified and characterized [13, 17, 20, 29]. The relationship between SOD and its defensive mechanism in fungi has been established by many studies.

Lentinus edodes is a kind of basidiomycetes, a family of fungi. Recently, it has been reported that mushroom contains various bioactive substances including immunostimulating activity, anticancer, or antibacterial substances as well as various types of proteases or carbohydrate-hydrolyzing enzymes, and so they are used in folk medicine and food [7, 27]. However, only a few studies on antioxidant enzymes such as SOD from basidiomycetes have been reported. The SODs purified from *Ganoderma microsporum* and *Pleurotus olearius* had a molecular weight of 98 kDa and 78 kDa, respectively, and the enzymes from both species are composed of four identical subunits and contain manganese in their active sites [26, 32].

In order to further understand the physiological function and application of SOD from basidiomycetes, it is important to know the molecular properties of the SOD from the mushroom family. Moreover, the information on SOD from *L. edodes* will enable to compare molecular

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structures and properties with those from different organisms including fungi and the diversity of fungal SODs. In this paper, we report the purification and characterization of SOD from fruiting bodies of the edible mushroom, *L. edodes*.

MATERIALS AND METHODS

Materials

Fruiting bodies of *L. edodes* were purchased from a local market in Kyongju, Korea. Cytochrome *c*, xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), Tris, BSA, EDTA, Bradford reagent, SDS, ammonium persulfate, acrylamide, *N,N'*-methylene-bis-acrylamide, *N,N,N',N'*-tetramethylethylenediamine, Coomassie Brilliant blue (CBB) R-250, and molecular weight marker proteins were obtained from Sigma Co. (St. Louis, U.S.A.). DEAE-Sepharose FF and Sephacryl S-200 were from Amersham Pharmacia Biotech (Uppsala, Sweden), and all other chemicals were of analytical grade.

SOD and Protein Assays

SOD activity was assayed by a modified method of McCord and Fridovich [30]. The reaction mixture (3 ml) contained the SOD preparation, 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.05 mM KCN, 0.01 mM cytochrome *c*, 0.05 mM xanthine, and a volume of xanthine oxidase sufficient for constant reduction of cytochrome *c* per min at 25°C. One unit (U) of SOD activity was defined as the amount that caused 50% inhibition of the rate of reduction of cytochrome *c*. Protein content was determined by the method of Bradford [6] using BSA as the standard.

Purification of SOD

All the purification steps were carried out at 4°C unless otherwise stated. Two kg of *L. edodes* was washed with distilled water and then homogenized in 4 l of 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA using a Waring blender. The homogenate was filtered through several layers of nylon cloth and centrifuged at 15,000 ×g for 30 min. The supernatant was brought to 40% saturation by addition of solid ammonium sulfate while stirring. The solution was stirred for an additional 3 h and then centrifuged at 15,000 ×g for 10 min. The resulting supernatant was brought to 85% saturation in ammonium sulfate, stirred for 3 h, and centrifuged again. The pellet was resuspended in 50 mM potassium phosphate buffer (pH 7.8) and dialyzed against the same buffer. The dialyzed solution was concentrated by ultrafiltration with YM-10 membrane (Amicon Co., Beverly, U.S.A.). The concentrated solution was applied to a DEAE-Sepharose FF column (2.6×30 cm) previously equilibrated in 50 mM potassium phosphate buffer (pH 8.3) and eluted with a linear gradient of NaCl (0 to 0.6 M) in the same buffer. The fractions with SOD activity were pooled and concentrated by ultrafiltration with YM-10 membrane. The concentrate was applied to a Sephacryl S-200 column (1.6×90 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.8) and eluted with the same buffer. The active fractions were pooled and concentrated. For further purification, preparative native PAGE and electroelution of protein with SOD activity were performed by the procedure described below.

Electrophoresis and Electroelution

SDS-PAGE was carried out using 12% polyacrylamide gel according to the method of Laemmli [25]. Nondenaturing PAGE was performed by the same method without SDS. Proteins were stained with 0.1% CBB R-250. SOD activity was located by staining the gel using the NBT reduction method according to the procedure of Beauchamp and Fridovich [4]. The protein band with the enzyme activity on the gel was sliced and electroeluted by Elutrap equipment (Schleicher & Schuell Inc., Keene, U.S.A.). For electroelution, the running buffer was the same as that used for native PAGE. Following electroelution, the sample was dialyzed against 50 mM potassium phosphate buffer (pH 7.8).

Determination of Molecular Weight

The molecular weight of the purified enzyme was determined by gel filtration chromatography on Sephacryl S-200 column (1.6×90 cm) according to the method of Andrews [2]. The molecular weight marker proteins used were cytochrome *c* (12.4 kDa), carbonic anhydrase (29 kDa), bovine albumin (66 kDa), and alcohol dehydrogenase (150 kDa). The enzyme and standard proteins were eluted with 50 mM potassium phosphate buffer (pH 7.8) and void volume of the column was determined by the elution of blue dextran. To determine the molecular weight of subunit in SOD, the enzyme was subjected to SDS-PAGE on the 12% gel. The molecular weight marker proteins (Bio-Rad Laboratories, Richmond, U.S.A.) used were phopshorylase B (102 kDa), BSA (78 kDa), ovalbumin (49.5 kDa), carbonic anhydrase (28.3 kDa), soybean trypsin inhibitor (28.3 kDa), and lysozyme (19.9 kDa).

Isoelectric Focusing (IEF)

The isoelectric point (pI) of the enzyme was determined by Mini IEF cell (Model 111, Bio-Rad Laboratories, U.S.A.) using 5% polyacrylamide gel and 2% ampholyte (pH 3.0 to 10.0) for 2 h under the final setting of 450 V and 2 W.

Amino Acid Analysis

The purified SOD was hydrolyzed in 6 N HCl at 110°C for 24 h. The amino acid composition of the enzyme was

analyzed with HPLC (Waters Co., Milford, U.S.A.) equipped with a Pico-Tag column (3.9300 mm) after phenylisocyanate derivatization. To determine the tryptophan content, the enzyme was directly digested with 4 M methanesulfonic acid and analyzed. Cysteine residues were oxidized to cysteic acid with a mixture of formic acid and hydrogen peroxide (19:1, v/v) and analyzed.

Metal Analysis

The purified SOD was dialyzed against deionized Milli-Q grade water for 24 h. The contents of each metal were determined by inductively coupled plasma (ICP) mass spectrophotometer (JY24, Jobin Ybon, France).

Effects of pH and Temperature on SOD Activity

To measure the effect of pH on SOD activity, the relative activity of the enzyme was determined at different pH ranges using 50 mM Tris-HCl for pHs 7.0 to 9.0, and 50 mM carbonate buffer for pHs 9.0 to 11.0. The temperature dependence of the enzyme activity was determined by measuring the relative activity at 20 to 80°C in 50 mM phosphate buffer (pH 8.0).

Effects of Inhibitors

To test the inhibitory activity of KCN, H_2O_2, and NaN_3 on the SOD, the purified enzyme was preincubated at 25°C for 30 min in 50 mM phosphate buffer (pH 7.8) containing each reagents and then enzyme activity was measured. The measurement of the SOD activity in the absence of inhibitors was carried out as the control.

Results and Discussion

Purification of SOD

The purification profile is summarized in Table 1. The enzyme was purified 420-fold to homogeneity with a specific activity of 546 U per mg of protein with a 6.5% yield. The elution profile of Sephacryl S-200 chromatography showed that the SOD activity eluted as a single peak (Fig. 1). As shown in Fig. 2A, the homogeneity of the purified preparation by nondenaturing PAGE showed a single protein band. Activity staining confirmed that the purified protein was responsible for the SOD from *L. edodes* (Fig. 2B).

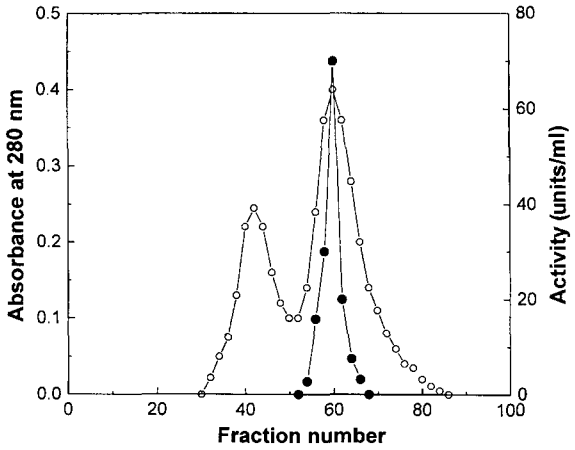

Fig. 1. Elution profile of SOD by gel filtration chromatography on Sephacryl S-200 column (1.6×90 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.8).
The enzyme was eluted with the same buffer. Flow rate was 0.2 ml/min and fraction volume was 2 ml. —○—, absorbance at 280 nm; —●—, SOD activity.

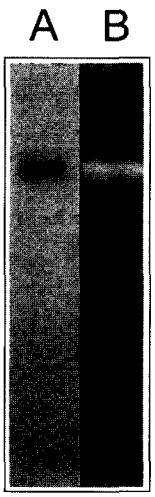

Fig. 2. Nondenaturing PAGE of SOD from *L. edodes*.
The concentration of the gel was 12%. (A) Protein was stained with CBB R-250. (B) Band with SOD activity was visualized by the method of Beauchamp and Fridovich [4].

Molecular Weight and Isoelectric Point

The molecular weight of the purified SOD was determined to be approximately 54 kDa by gel filtration on Sephacryl S-200 column (Fig. 3A). The molecular weight of the SOD

Table 1. Purification of the SOD from *Lentinus edodes*.

Purification step	Total protein (mg)	Total activity (U)[a]	Specific activity (U/mg)	Recovery (%)	Purification fold
Crude extract	3,126.0	4,200	1.3	100.0	1.0
Ammonium sulfate precipitation	800.1	2,898	3.6	69.0	2.8
DEAE Sepharose FF	67.3	1,764	26.3	42.0	20.2
Sephacryl S-200	7.6	1,256	165.3	29.9	127.2
Preparative PAGE	0.5	273	546.0	6.5	420.0

[a]One unit is defined as the amount of SOD required to cause a 50% inhibition of the rate of reduction of cytochrome *c* at 25°C.

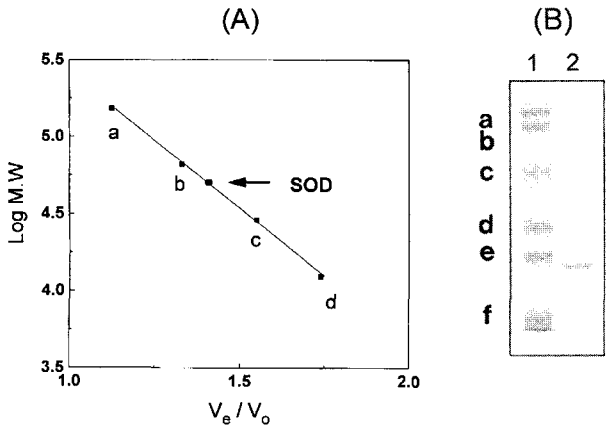

Fig. 3. Determination of molecular weight of SOD and its subunit.

(A) Molecular weight determination by Sephacryl S-200 gel filtration chromatography. V_e is the elution volume of each protein and V_o is the void volume of the column. Standard proteins used were: a, alcohol dehydrogenase (150 kDa); b, albumin bovine (66 kDa); c, carbonic anhydrase (29 kDa); and d, cytochrome *c* (12.4 kDa). (B) SDS-PAGE of SOD on 12% gel. Lane 1, molecular weight markers: a, phosphorylase B (102); b, BSA (78 kDa); c, ovalbumin (49.5 kDa); d, carbonic anhydrase (28.3 kDa); e, soybean trypsin inhibitor (28.3 kDa) and f, lysozyme (19.9 kDa). Lane 2: the purified SOD.

subunit was estimated to be about 27 kDa by SDS-PAGE in the presence of β-mercaptoethanol (Fig. 3B), indicating that the SOD from *L. edodes* is probably composed of two identical subunits. The molecular weight of SODs is diverse depending on their sources, and most SODs are usually homodimeric or homotetrameric proteins [14]. In general, fungal SOD has a molecular weight in the range of 40 kDa to 140 kDa, and is composed of subunits with molecular mass of 20 kDa to 26 kDa [15, 18, 20]. The SODs from *G. microsporum* and *P. olearius* has a molecular weight of 98 kDa and 78 kDa, respectively, and the SODs from both sources composed of four identical subunits with molecular masses of 25 kDa and 20 kDa, respectively [26, 32]. The above findings indicate that the SOD of *L. edodes* is smaller than those of other basidiomycetes. The pI value of the purified enzyme was 4.9 on 5% polyacrylamide gel by IEF (Fig. 4). The result revealed that only one isoform is present in *L. edodes*. In contrast, the SOD from *G. microsporum* was separated into two isoforms on IEF gel with the pI values of 6.34 and 5.06, respectively [32]. The pI values of the SODs from *A. niger* and *A. flavus* were 5.0 and 5.7, respectively [18]. It has been reported that SOD from *Mycobacterium bovis* had an acidic value of pI 4.5 [21]. The result indicated that the pI value of the *L. edodes* SOD is similar to pI 5.06 of the isoform of *G. microsporum* SOD, pI 5.0 of the enzyme of *A. niger*, and pI 5.05 of *C. limonium* [1]. On the other hand, the pI of the SOD is lower than those of *C. neoformans* var. *neoformans* SOD isoenzymes which have pIs of 5.9, 6.15, 6.35, and 6.6, respectively [15].

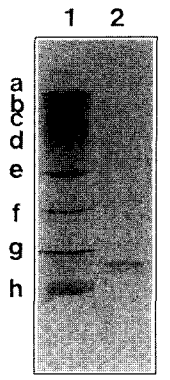

Fig. 4. Isoelectric focusing analysis of purified SOD by Mini IEF cell ((Model 111, Bio-Rad Co. U.S.A.) using 5% polyacrylamide gel and 2% ampholyte (pH 3.0 to 10.0).

Isoelectric point markers used were a, cytochrome *c* (9.6); b, lentil lectin (8.2, 8.0, 7.8); c, human hemoglobin (7.1); d, equine myoglobin (7.0, 6.8); e, human carbonic anhydrase (6.5); f, bovine carbonic anhydrase (6.0); g, β-lactoglobulin B (5.1); and h, phycocyanin (4.75, 4.65, 4.45).

Metal Contents of the Enzyme

The purified SOD was found to contains 0.89 g-atom of iron, 0.75 g-atom of zinc, and 0.46 g-atom of copper per mol of enzyme as shown in Table 2. Small amounts of nickel were detected in the enzyme preparation from *L. edodes*, but manganese was not detected. Generally, it has been reported that most fungal SODs contain Cu/Zn or Mn in their active site. The SODs from *Cryptococcus* species and *Aspergillus* species are CuZnSOD [15, 18] and those of *G. microsporum* and *Actinomyces* species are MnSOD [3, 32]. In contrast, *C. albicans* has both types of SODs [20, 34]. However, some SODs have been reported to contain various metals in the enzyme. For example, the SOD from *Ginkgo biloba* contains 1.37 g-atoms of iron, 0.22 of manganese, 2.24 of copper, and 2.33 of zinc per mol of enzyme [9], and that of *Halobacterium halobium* contains 1.53 atoms of manganese, 0.5 of zinc, 1.54 of copper per molecule [33]. In addition, the SODs from *Methanobacterium bryantii* and *Thermoplasma acidophilum* contain both iron and zinc [23, 36]. It has been suggested that zinc in these enzymes is not functional but occupies partial sites of iron or extraneous sites on the enzyme [23]. On the basis of this result, the iron-containing SOD from *L. edodes* is comparable to *G. microsporum* SOD

Table 2. Metal contents of SOD from *L. edodes*.

Metal	Metal content	
	(μg/mg enzyme)	(g-atom/mol enzyme[a])
Nickel	0.02	0.02
Iron	0.92	0.89
Zinc	0.91	0.75
Copper	0.54	0.46
Manganese	-	-

[a]Calculation was based on a molecular weight of 54 kDa.

containing manganese in its active site. It is well known that the MnSOD and FeSOD are structurally similar. Therefore, it is likely that the SODs from basidiomycetes, *L. edodes,* and *G. microsporum* might have structural homology.

Effects of Inhibitors on SOD Activity

A number of potential enzyme inhibitors are shown in Table 3. H_2O_2 at the concentration of 5 mM inhibited 55% of the enzyme activity, however, KCN and NaN_3 had little inhibitory effect on the enzyme activity. It is known that CuZnSOD is sensitive to both KCN and H_2O_2. FeSOD is sensitive to H_2O_2 only, whereas MnSOD is insensitive to both inhibitors, but is inhibited by azide [16]. The inhibitory pattern of the inhibitors on SOD from *L. edodes* was similar to that of FeSOD. These results may suggest that the SOD from *L. edodes* might be FeSOD. This information is in good agreement with the result of metal analysis of the enzyme, and indicates that iron in SOD plays an important role in enzymatic catalysis, and zinc and copper may represent either partial occupancy of iron sites or the stabilization of the active site.

Table 3. Effects of specific inhibitors on the SOD activity.

Inhibitor	Relative activity (%)		
	0.1 mM	1 mM	5 mM
None	100.0	100.0	100.0
KCN	98.2	90.6	89.3
H_2O_2	90.8	75.5	45.0
NaN_3	98.3	92.7	90.6

Table 4. Amino acid compositions of the iron-containing SODs from various sources.

Amino acid	Residues/subunit[a]			
	L. edodes	*C. limonum* [1]	*G. biloba* [10]	*E. coli* [37]
Cys	5	3	1	1
Asx[b]	14	27	22	22
Glx[c]	24	18	21	16
Ser	17	20	18	10
Gly	25	23	36	16
His	6	3	4	6
Arg	9	7	4	4
Thr	16	12	12	13
Ala	22	18	32	26
Pro	39	12	11	9
Tyr	1	6	2	6
Val	16	14	12	11
Met	1	2	4	0
Ile	18	8	11	8
Leu	25	18	16	15
Phe	10	6	6	10
Trp	1	3	3	4
Lys	10	15	18	10
Total	259	215	233	187

[a]Calculation was based on a molecular weight of 27 kDa.
[b]Sum of aspartic acid and asparagine.
[c]Sum of glutamic acid and glutamine.

Amino Acid Composition

The amino acid composition of the SOD from *L. edodes* was compared with those of iron-containing SODs from various sources (Table 4). One of the iron-containing SODs show high content of glutamic acid/glutamine, glycine, alanine, and leucine, but with low amount of methionine, cysteine, tyrosine, and tryptophan. Amino acid composition of *L. edodes* SOD was shown to be similar to those of other iron-containing SODs [1, 10, 38]. The SOD, however, contains a relatively large amount of glutamic acid/

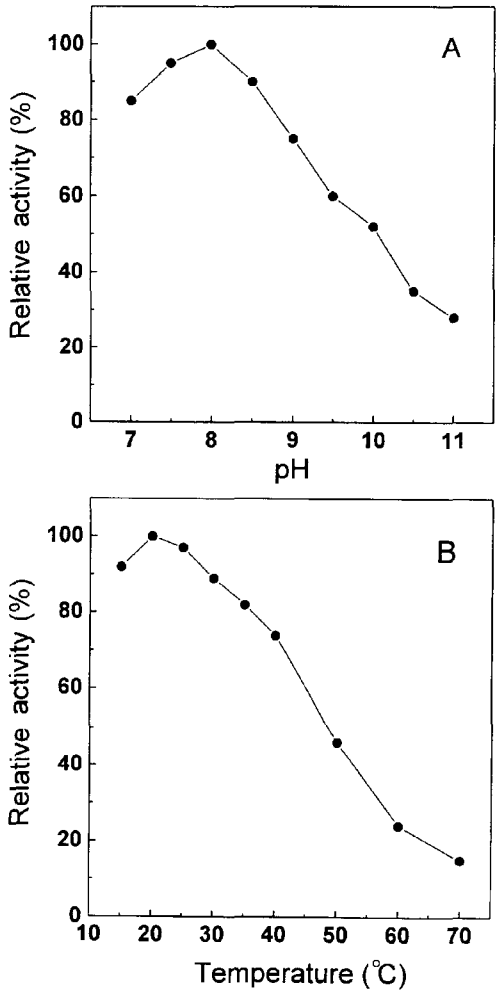

Fig. 5. The effects of pH and temperature on the SOD activity. (A) The following buffer systems were used: pH 7.0–9.0 Tris-HCl (50 mM) and pH 9.0 and 11.0 carbonate buffer (sodium carbonate-sodium bicarbonate, 50 mM). (B) The effect of temperature on the SOD activity. The enzyme reaction was performed in potassium phosphate buffer (pH 8,0).

glutamine, proline, cysteine, isoleucine, and leucine, but only a small amount of aspartic acid/asparagine, tyrosine, and tryptophan compared with other iron-containing SODs. On the basis of these results, it can be said that *L. edodes* SOD has a high degree of homology with the Fe-SOD from other sources.

Effects of pH and Temperature on SOD Activity

The effect of pH on the activity of SOD from *L. edodes* is shown in Fig. 5A. The enzyme showed maximum activity at pH 8.0 in the range of pHs 7.0 to 11.0. The effect of pH below 7 was not assayed because xanthine oxidase is inactive below pH 6.5. The pH dependence of xanthine oxidase above pH 7.0 was effected by varying the amount of xanthine oxidase added to each assay. The optimum temperature for the enzyme was found to be 20°C (Fig. 4B). The pH and temperature dependences of the SODs from some fungi were investigated. For example, the SOD from *Aspergillus* species retained maximal activities in the range of pHs 7.0 to 10.0, with highest activity at 20°C compared with that at 37°C [18]. The SOD from *Cryptococcus* species showed similar dependence [15]. In addition, Almansa *et al.* [1] had reported that FeSOD from *Citrus limonum* showed a maximum activity at pH 7.8. and Hosono and Satake [19] had reported that SOD from *Latococcus lactis* had its optimal activity in the neutral pH range of 6.5 to 8.0. The SOD from *Aerobacter aerogenes* [22] was stable up to 35°C at pH 7.0 and the enzyme from *Bacillus circulans* [28] was stable at pH 7.0 and 20°C. From the above, the SOD from *L. edodes* had a similar pH and temperature dependence with those from other sources.

Acknowledgments

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References

1. Almansa, M. S., L. A. del Rio, and F. Sevilla. 1994. Characterization of an iron-containing superoxide dismutase from a higher plant, *Citrus limonum. Physiol. Plant* **90:** 339-347.
2. Andrews, P. 1965. The gel filtration behavior of proteins related to their molecular weight over a wide range. *J. Biochem.* **96:** 595-606.
3. Barkley, K. B. and E. M. Gregory. 1990. Tetrameric manganese superoxide dismutases from anaerobic *Actinomyces. Arch. Biochem. Biophys.* **280:** 192-200.
4. Beauchamp, C. and I. Fridovich. 1971. Superoxide dismutase: Improved assays and an applicable to acrylamide gels. *Anal. Biochem.* **44:** 276-287.
5. Bowler, C., M. Van Montagu, and D. Inze. 1992. Superoxide dismutase and stress tolerance. *Annu. Rev. Physiol. Plant Mol. Biol.* **43:** 83-116.
6. Bradford, M. M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248-254.
7. Chang, S. T. and P. G. Miles. 1989. *Edible Mushrooms and Their Cultivation,* pp. 35-40. CRC Press, FL, U.S.A.
8. Chang, W.-S. and J.-S. So. 1998. False positive SOD activity of *Bifidobacterium* spp. grown in MRS medium. *J. Microbiol. Biotechnol.* **8:** 305-309.
9. del Rio, L. A., F. Sevilla, L. M. Sandalio, and J. M. Palma. 1991. Nutritional effect and expression of SODs: Induction and gene expression; diagnostics; prospective protection against oxygen toxicity. *Free Rad. Res. Commun.* **12-13:** 819-828.
10. Duke, M. V. and M. L. Salin. 1985. Purification and characterization of an Iron-Containing Superoxide Dismutase from a Eukaryote, *Ginkgo Biloba. Arch. Biochem. Biophys.* **243:** 305-314.
11. Fridovich, I. 1978. The biology of oxygen radicals. *Science* **20:** 875-880.
12. Fridovich, I. 1995. Superoxide radicals and Superoxide dismutases. *Annu. Rev. Biochem.* **64:** 97-112.
13. Goscin, S. A. and I. Fridovich. 1972. The purification and properties of superoxide dismutase from *Saccharomyces cerevisiae. Biochim. Biophys. Acta* **289:** 276-283.
14. Halliwell, B. and John M. C. Gutteridge. 1998. *Free Radicals in Biology and Medicine* (2nd ed.) pp. 106-123. Clarendon. Oxford, U.K.
15. Hamilton, A. J. and M. D. Holdom. 1997. Biochemical comparison of the Cu, Zn Superoxide dismutases of *Cryptococcus neoformans var. neoformans* and *Cryptococcus neoformans var. gattii. Infec. Immun.* **65:** 488-494.
16. Hassan, H. M., H. Pougherty, and I. Fridovich. 1980. Inhibitors of Superoxide dismutase: A cautionary tale. *Arch. Biochem. Biophys.* **199:** 349-354.
17. Holdom, M. D. and A. J. Hamilton. 1995. Purification, N-terminal amino acid sequence and partial characterization of a Cu, Zn superoxide dismutase from the pathogenic fungus *Aspergillus fumigatus. Free Rad. Res.* **22:** 519-531.
18. Holdom M. D., R. J. Hay, and A. J. Hamilton. 1996. The Cu,Zn Superoxide dismutase of *Aspergillus flavus, Aspergillus niger, Aspergillus nidulans,* and *Aspergillus terreus*: Purification and biochemical comparison with the *Aspergillus fumigatus* Cu, Zn Superoxide dismutase. *Infec. Immun.* **64:** 3326-3332.
19. Hosono, A. and N. Satake. 1993. Isolation and characterization of superoxide dismutase from *Lactococcus lactis* subsp. cremoris R-48. *Anim. Sci. Technol.* **64:** 599-604.
20. Hwang, C.-S., G. Rhie, S.-T. Kim, Y.-R. Kim, W.-K. Huh, Y.-U. Baek, and S.-O. Kang. 1999. Copper- and zinc-containing superoxide dismutase and its gene from *Candida albicans. Biochim. Biophys. Acta* **1427:** 245-255.

21. Kang, S.-K., Y.-J. Jung, C.-H. Kim, and C.-H. Song. 1998. Extracellular and cytosolic iron superoxide dismutase from *Mycobacterium bovis* BCG. *Clin. Diagn. Lab. Immunol* **5:** 784-789.
22. Kim, S.-W., S.-O. Lee, and T.-H. Lee. 1991. Purification and characterization of superoxide dismutase from *Aerobacter aerogenes*. *Agric. Biol. Chem.* **55:** 101-108.
23. Kirby, T. W., J. R. Jr., Lancaster, and I. Fridovich. 1981. Isolation and characterization of the iron-containig superoxide dismutase of *Methanobacterium bryantii*. *Arch. Biochem. Biophys.* **210:** 140-148.
24. Kroll, J. S., P. R. Langford, and B. M. Loynds. 1991. Copper-zinc superoxide dismutase of *Haemophilus influenza* and *H. parainfluenza*. *J. Bacteriol.* **173:** 7449-7457.
25. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680-685.
26. Lavelle, F. and A. M. Michelson. 1975. Purification and study of two superoxide dismutase of the mushroom *Pleurotus olearius*. *Biochimie* **57:** 375-381.
27. Lee, J.-H., S.-M. Kin, H.-M. Kim, N.-D. Hong, and I.-D. Yoo. 1997. Immunostimulating activity of polysaccharides from mycelia of *Phellinus linteus* grown under different culture conditions. *J. Microbiol. Biotechnol.* **7:** 52-55.
28. Lee, T.-H. and S.-O. Lee. 1988. Purification and properties of superoxide dismutase from *Bacillus circulans*. *Agric. Biol. Chem.* **52:** 1361-1367.
29. Lerch, K. and E. Schenk. 1985. Primary structure of copper zinc superoxide dismutase from *Nerospora crassa*. *J. Biol. Chem.* **260:** 9559-9566.
30. McCord, J. M. and I. Fridovich. 1969. Superoxide dismutase: An enzymic function for erythrocuprein. *J. Biol. Chem.* **244:** 6049-6055.
31. Okada, S., S. Kanematsu, and K. Asada. 1979. Intracellular distribution of manganese and ferric superoxide dismutase in blue-green algae. *FEBS Lett.* **103:** 106-110.
32. Pan, S. M., J. S. Ye, and R. S. Hseu. 1997. Purification and characterization of manganese superoxide dismutase from *Ganoderma microsporum*. *Biochem. Mol. Biol. Int.* **42:** 1035-1043.
33. Rabinowitch, H. D. and I. Fridovich. 1983. Superoxide radicals, superoxide dismutases and oxygen toxicity in plants. *Photochem. Photobiol.* **37:** 679-690.
34. Rhie, G., C.-S. Hwang, M. J. Brady, S.-T. Kim, Y.-R. Kim, W.-K. Huh, Y.-U. Baek, B.-H. Lee, J.-S. Lee, and S.-O. Kang. 1999. Manganese-containing superoxide dismutase and its gene from *Candida albicans*. *Biochim. Biophys. Acta* **1426:** 409-419.
35. Salin, M. L. and D. Oesterhelt. 1988. Purification of a manganese-containing superoxide dismutase from *Halobacterium halobium*. *Arch. Biochem. Biophys.* **260:** 806-810.
36. Searcy, K. B. and D. C. Searcy. 1981. Superoxide dismutase from the Archaebacterium *Thermoplasma acidophilum*. *Biochim. Biophys. Acta* **670:** 39-46.
37. Shin, S.-Y. and J.-H. Park. 1997. Activities of oxidative enzymes related with oxygen tolerance in *Bifidobacterium* sp. *J. Microbiol. Biotechnol.* **8:** 305-309.
38. Yost, F. J. and I. Fridovich. 1973. An iron-containing superoxide dismutase from *Escherichia coli*. *J. Biol. Chem.* **248:** 4905-4908.