Preliminary

Isolation and Characterization of Manganese (II) Peroxidase (MnP) Produced by *Pleurotus ostreatus*

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(Received May 31, 1997)

Keywords : Lignin degrading enzyme, Manganese (II) peroxidase, Laccase, White-rot fungi, *Pleurotus ostreatus*

White-rot fungi produce lignin-degrading enzymes including lignin peroxidase (LiP), manganese (II) peroxidase (MnP) and laccase. These enzymes can be applied to conversion of lignocellulosic biomass to useful materials, degradation of chemicals which cause environmental pollution and other economically important processes. *Pleurotus ostreatus*, which has been cultivated widely in Japan as an edible mushroom, degrades lignin efficiently. This fungus has been found to produce lignin degrading enzymes extracellularly in both liquid and solid cultures. This study aims to isolate and characterize MnP produced by *P. ostreatus*.

1. Production of lignin degrading enzymes by *P. ostreatus*

In the culture of *P. ostreatus* IS-1, addition of hot water extracts of wheat bran to a glucose-peptone medium consisted of 2% glucose, 0.5% polypeptone, 0.2% yeast extract, 0.1% KH_2PO_4 and 0.05% $MgSO_4 \cdot 7H_2O$ enhanced extensively the production of MnP and laccase. Alkaline extracts of wheat bran also enhanced laccase production. The enzyme activities in these media were found to be much higher than those in Kirk's media. LiP activity was not detected in the conditions examined.

Except for *P. ostreatus* IFO 30776, strains of *P. ostreatus* (IS-1, 48 and K-2946) produced high activity of MnP up to 40 units per ml of the medium in 10 to 14 days of incubation. In these cultures, production of laccase started prior to the production of MnP, suggesting expression of laccase was dependent on mycelial growth.

Ion exchange chromatography of the culture concentrate of strain 48 using a column of DEAE-Sepharose CL-6B gave two peaks of MnP activity as shown in Fig. 1. An elution

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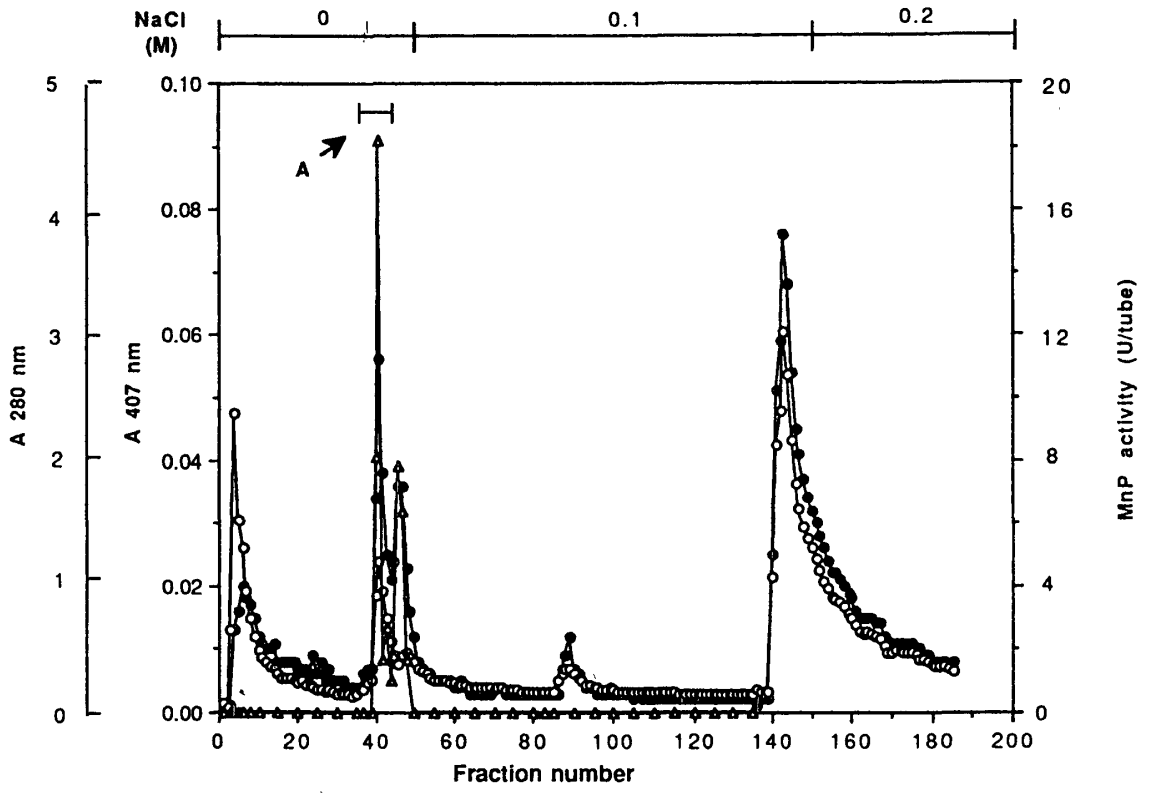

Fig. 1. Separation of MnP of *P. ostreatus* strain 48 by DEAE-Sepharose chromatography. MnP activity was determined using guaiacol as a substrate. A, fraction which was subjected to further purification. —○—, absorbance at 407 nm; —●—, absorbance at 280 nm; —△—, MnP activity.

pattern of this enzyme was similar to those of other strains examined. HPLC using a Mono Q HR 10/10 column and IEF gel electrophoresis using an Ampholine PAG plate (pH 3.5–9.5, Pharmacia) showed at least 4 different MnP proteins were produced.

Table 1. Comparison of amino acid sequences of N-terminal of MnP from *P. ostreatus* and other white-rot fungi

Pleurotus ostretaus (IS-1) MnP-3*	ATCADGRTTA-NAACXVLF-P
Pleurotus ostretaus (IS-1) MnP-4*	ATCADGRXTA-NAPXXVLF-X
Pleurotus ostretaus (K-2946) MnP-3*	ATCADXXTXA-NXQXXV
Pleurotus ostretaus (No. 48) MnP-3*	ATCADGRXTA-NAACXXVC-P
Pleurotus ostretaus (IFO 30776) MnP-3*	ATCADGRTTA-NAACXVLF-P
Pleurotus ostretaus (IFO 30162) MnP[1)]	ATCAGGQVTA-NAACCVLF-S
Pleurotus eryngii MnP-1[2)]	ATDADGRTTA-NAACCVLF-S
Pleurotus eryngii MnP-1[2)]	ATDDDGRTTA-DAACCILF-X
Pleurotus pulmonarius MnP-2[3)]	ATCADGRTTA-NAACCVLF-P
Pleurotus chrysosporium MnP-1[4)]	AVCPDGTRV-SHAACCA-FIP
Lentinula edodes MnP-1[5)]	AVGSDGTVVP-DSVQYD-FIP

* This work.

2. N-terminal amino acid analysis of MnP

Proteins separated by IEF gel electrophoresis were transferred to polyvinydilenefluoride (PVDF) membranes by electroforesis. Proteins on the membrane were treated with *S*-pyridylethylation to protect cystein residues from decomposition, followed by amino acid analysis by a protein sequencer (Applied Biosystems, Model 491). As shown in Table 1, these enzymes showed high similarity in N-terminal amino acid sequence with that of other strains of *P. ostreatus*[1] as well as that of MnP isolated from *P. eryngii*[2] and *P. pulmonarius*[3] However, the sequences were found to be different from those of MnPs from *Phanerochaete chrysosporium*[4] and *Lentinus edodes*[5].

Acknowledgement

The authors are indebted to Dr. T. Hayashi for his kind suggestion on the sequence analysis of MnP.

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