Properties of Mycelial Aggregate-specific Lectin of *Pleurotus cornucopiae* Produced in *Pichia pastoris*

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CDNA of a mycelial aggregate-specific lectin of *Pleurotus cornucopiae* was expressed in *Pichia pastoris*, and the expression product was purified and characterized. The product was functional, and the hemagglutinating activity was inhibited most strongly by the addition of N-acetyl-D-galactosamine as was the native lectin. The native lectin is a glycoprotein having five glycosylation recognition signals, and the expression product showed slightly larger molecular mass than that of the native one due to further glycosylation.

Key words: expression in *Pichia pastoris*; glycosylation of lectin; mycelial aggregate-specific lectin; *Pleurotus cornucopiae*

Lectins are proteins or glycoproteins that bind carbohydrates specifically and reversibly. While lectins have been found in various organisms including fungi, little is known about the in vivo functions of lectins in organisms that produce them. 1–3) We have reported that in *Pleurotus cornucopiae* two kinds of lectins were synthesized in a developmental stage-specific manner, one in fruitbodies and the other in mycelial aggregates. 4–7) The lectin from the mycelial aggregates (PCL-M) is active when the 40-kDa subunit forms a multimer through a disulfide bridge in the presence of Ca\(^{2+}\).6) We cloned the cDNA of the PCL-M subunit, and sequenced (Sumisa, F., et al., unpublished results). PCL-M is composed of 373 amino acids, thirty-three of which constitute a signal sequence. The cDNA was expressed in *Escherichia coli* and a product cross-reactive with the anti-PCL-M serum was obtained. However, due to a lack of glycosylation the expression product was insoluble and formed inclusion bodies, so that the hemagglutinating activity could not be determined. It is known that in the amino acid sequence of PCL-M, five glycosylation recognition signals (2\(NPT\), 20\(NIS\), 47\(NIT\), 95\(NPS\), and 288\(NQT\)) are included, and PCL-M is expressed specifically in a solid-state culture. In this study we attempted to use another gene expression system of the methylotrophic yeast *Pichia pastoris*, in which proteins are glycosylated.

*Pichia pastoris* GS115 (his4-) and plasmid pPIC9K were purchased from Invitrogen (Carlsbad, USA). Since PCL-M has the signal sequence of 33 amino acid residues, cDNA encoding the mature PCL-M was prepared by PCR using primers *Xho*I-F (CTCGAGAAGAACTAATCCGACTTGCAAG) and *Not*I-R (GCGGCCGCTTAGGCTGGTGG). Expression plasmid was constructed by inserting the cDNA for the mature PCL-M into the *Xho*I-*Not*I site of pPIC9K. The plasmid was linearized at the *his*4 gene by *Sal*I, and introduced into *P. pastoris* GS115 (his4-) by electroporation using the Gene Pulser (Bio-Rad, USA). Among the His+ transformants chosen, G418-resistant colonies were selected on plates containing 1% yeast extract, 2% peptone, 2% dextrose, and 6 mg/ml G418. One of the transformants was cultured in a 3-liter table-top glass fermenter (Model TS-M; Takasugi Seisakusho, Tokyo) using basal salts as the medium with the addition of methanol as a carbon source and an inducer for gene expression as reported. 8)

Since PCL-M is known to have an affinity toward porcine stomach mucin (PSM), 6) it was purified by a batch-wise affinity chromatography using PSM as a ligand. PSM was coupled with *N*-hydroxysuccinimide-activated Sepharose (Amersham Biosciences). The culture fluid (5 ml) was defatted by extraction with 1-butanol and concentrated to 5-fold by ultra-filtration through a membrane (Amicon Ultra-4, Millipore), and the proteins were dissolved in Tris-buffered saline, pH 7.4, containing 50 mM Ca\(^{2+}\) (TBS-Ca). We cloned the cDNA of the PCL-M subunit, and sequenced (Sumisa, F., et al., unpublished results). PCL-M is composed of 373 amino acids, thirty-three of which constitute a signal sequence. The cDNA was expressed in *Escherichia coli* and a product cross-reactive with the anti-PCL-M serum was obtained. However, due to a lack of glycosylation the expression product was insoluble and formed inclusion bodies, so that the hemagglutinating activity could not be determined. It is known that in the amino acid sequence of PCL-M, five glycosylation recognition signals (2\(NPT\), 20\(NIS\), 47\(NIT\), 95\(NPS\), and 288\(NQT\)) are included, and PCL-M is expressed specifically in a solid-state culture. In this study we attempted to use another gene expression system of the methylotrophic yeast *Pichia pastoris*, in which proteins are glycosylated.

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Protein contents were estimated by Bradford’s method.9) The expression product was soluble in TBS-Ca, and after abolishing EDTA, it showed hemagglutinating activity toward rabbit red blood cells. The activity was assayed as reported.8) As shown in Table 1, PCL-M was purified 20-fold in the single step.

When the culture fluid of the transformant bearing cDNA for the mature PCL-M was subjected to SDS-PAGE and immunoblotted with the anti-PCL-M serum, a band having about 42 kDa was obtained (lane 1 of Fig. 1A and B). After passage through PSM-Sepharose, the lectin was purified to give a single band (lane 2). The molecular mass of the expression product was larger than that of the native PCL-M, which is known as a glycoprotein6) (lane 3). As the expression product of PCL-M cDNA in E. coli gave approximately 36 kDa (lane 4), which is not glycosylated, the product in P. pastoris must be heavily glycosylated.

As shown in Table 2, the hemagglutinating activity of the expression product was inhibited most strongly by N-acetyl-d-galactosamine, and it was also inhibited by d-galactose, d-fucose, and lactose but not by N-acetyl-d-glucosamine, as was the native PCL-M.6) These results indicate that although glycosylation occurred more than in the case of the native PCL-M, the expression product in P. pastoris is functional. Further studies using this product on the role of PCL-M in the process of fruitbody formation are in progress.

### References