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A Novel Developmental Stage-Specific Lectin of the Basidiomycete *Pleurotus cornucopiae*

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A novel lectin was isolated from mycelia of the basidiomycete *Pleurotus cornucopiae* grown on solid medium. The lectin was purified to homogeneity by mucin-Sepharose affinity chromatography. The molecular mass of the lectin was 40 kDa under reducing conditions, but the subunits were polymerized through disulfide bridges under physiological conditions. Hemagglutinating activity of this lectin was completely inhibited by 2-mercaptoethanol, indicating that the multimer is active. The activity was also inhibited by EDTA, and restored by $CaCl_2$. *N*-Acetyl-D-galactosamine was the most potent hapten inhibitor. N-terminal amino acid sequence analysis revealed that the mycelial lectin was different from the fruit body lectin of this organism. The mycelial lectin appeared prior to fruit body formation and disappeared during the formation of fruit bodies. The lectin was localized on the surface of solid-medium-grown mycelia, and only dikaryotic, and not monokaryotic, mycelia produced the lectin. These results suggest that the appearance of this lectin is associated with fruit body formation.

Lectins are carbohydrate-binding proteins that are found in various organisms (21). Although there have been several reports of fungal lectins found in fruit bodies (8–10, 13, 18, 23, 24), there are few reports of mycelial lectins (17), and their physiological functions in nature have not been completely explained yet. We have found a new lectin in the fruit body of *Pleurotus cornucopiae* and characterized it (8, 24). This lectin was not contained in vegetatively growing mycelia in liquid medium, but it appeared after the onset of fruit body formation on solid medium (8). In the course of studies on the function of this lectin, we found and established two strains of *P. cornucopiae*, named KC-1 and KC-2, with respect to the lectin content in their fruit bodies (14, 15).

In this study, we found that mycelia of *P. cornucopiae* grown on solid medium exhibit hemagglutinating activity and that the active substance is different from the lectin found in the fruit body. We describe here the purification and properties as well as localization and developmental stage-specific expression of this novel mycelial lectin, suggesting that the lectin participates in the process of fruit body formation in this organism. The results presented in this paper together with those of our other studies (8, 24) demonstrate that two lectins are produced in *P. cornucopiae* in a developmental stage-specific manner.

MATERIALS AND METHODS

Organisms. Two strains of *P. cornucopiae*, KC-1 and KC-2, were studied. The former contains the fruit body lectin (PCL-F), but the latter does not (15, 24). Dikaryotic mycelia of these strains were maintained on agar slants of yeast extract-malt extract-glucose medium (8). Monokaryotic mycelia were obtained by single-spore isolation.

Fruit body formation. Dikaryotic mycelia on the slant were first grown in liquid yeast extract-malt extract-glucose medium at 28°C for 7 days with shaking (stage 0). The liquid-medium-grown mycelia were inoculated onto a solid medium composed of sawdust and rice bran (8) and incubated at 25°C for 20 days in the dark (stage I). At this time, mycelia grew all over the surface of the medium. To stimulate fruit body formation, the surfaces of the mycelia were scraped and the cultures were transferred to 20°C in the light. After 4 to 5 days primordia or immature fruit bodies appeared (stage II), and after further 5 to 6 days mature fruit bodies were harvested (stage III). In this study, growth of *P. cornucopiae* were divided into four phases (0, I, II, and III), and the developmental process was sectioned into three stages (I, II, and III) as indicated above. "Liquid medium" in this article refers to the yeast extract-malt extract-glucose medium, and "solid medium" is sawdust-rice bran medium.

Purification. Mycelia (fresh weight, 100 g) of strain KC-2 grown on the solid medium for 20 days at 25°C in the dark were harvested by scraping mycelia off the surface and homogenized in a blender for 10 min with 200 ml of Tris-buffered saline (TBS) (10 mM Tris-HCl and 150 mM NaCl, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride (Sigma). The homogenate was centrifuged at 10,000 × *g* for 20 min, and the precipitate was homogenized again in 150 ml of the same buffer. The extracts were combined, and solid ammonium sulfate was added to 80% saturation. The precipitate was collected by centrifugation at 10,000 × *g* for 20 min and dissolved in 30 ml of TBS. The solution was

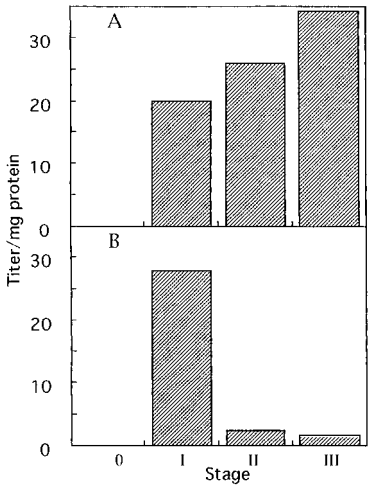

FIG. 1. Appearance of lectin activity at four growth phases of *P. cornucopiae*. Crude extracts prepared from *P. cornucopiae* KC-1 (A) and KC-2 (B) at each of the four growth phases were assayed for hemagglutinating activity.

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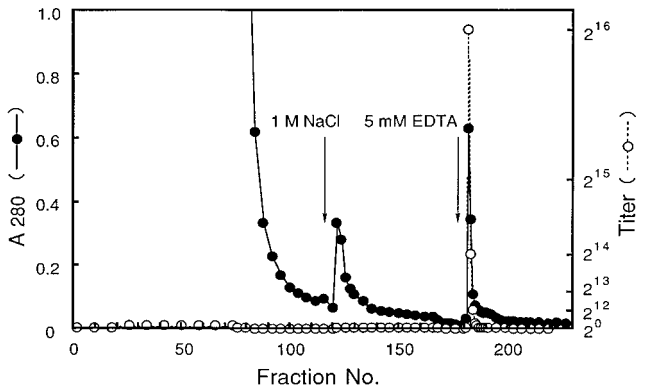


FIG. 2. Purification of PCL-M by affinity chromatography. Ammonium sulfate precipitates of the crude extract prepared from the stage I mycelia of *P. cornucopiae* KC-2 were dissolved in TBS and loaded onto a PSM-Sepharose 4B column. At the points indicated by arrows, the elution buffer was changed to 1 M NaCl and then to 5 mM EDTA in TBS. Fractions of 0.7 ml per tube were collected, monitored by A_{280}, and examined for hemagglutinating activity.

loaded onto an affinity column (0.6 by 3.5 cm) which was prepared as follows. Porcine stomach mucin (PSM) (Sigma) was conjugated to CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's instructions. The column was washed with TBS and 1 M NaCl in TBS to remove unbound proteins. Specifically bound protein was eluted with 5 mM EDTA in TBS. $CaCl_2$ was added to the EDTA-eluted fraction to a final concentration of 50 mM in order to restore the activity. The protein content was estimated by the method of Bradford (2).

Hemagglutination assay. The lectin activity was titrated by serially diluting the sample (20 μl) with assay solution (1 mg of bovine serum albumin per ml and 50 mM $CaCl_2$ in TBS) and then mixing it with an equal volume of a 2% suspension of rabbit erythrocytes. The hemagglutination units (titer) were calculated as the reciprocal of the multiple of the dilution giving a positive reaction after 1 h at room temperature (12). For inhibition assays, lectin solution (titer 8) was incubated with the test sugar, which was serially diluted with TBS. D-Fucose, L-fucose, D-fructose, D-galactose, D-glucose, D-mannose, D-galactosamine, and saccharose were purchased from Kanto Chemicals (Tokyo, Japan); D-ribose, D-xylose, D-glucosamine, *N*-acetyl-D-glucosamine, lactose, maltose, and raffinose were from Wako Pure Chemicals (Osaka, Japan); and *N*-acetyl-D-galactosamine, melibiose, and the tetramer of *N*-acetyl-D-galactosamine were from Funakoshi (Osaka, Japan). *N*-Acetyl-lactosamine, mucins derived from bovine submaxillary gland (BSM), PSM, asialo-BSM, fetuin, asialo-fetuin, and thyroglobulin were obtained from Sigma Chemicals (St. Louis, Mo.).

Electrophoresis and staining. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) proceeded as described by Laemmli (11) on 15% or 4 to 15% gradient gels (Daiichi Kagaku, Tokyo, Japan) in the presence or absence of 2-mercaptoethanol (2-ME). After each run, protein bands were stained with silver (Kanto Chemicals) or Coomassie brilliant blue R-250 (Merck). Carbohydrates were visualized with the periodic acid-Schiff stain (19). Sigma kits (MW SDS 70L and 200) were used as size standards.

Immunoblotting. To prepare antiserum, a rabbit was immunized by a subcutaneous injection of electrophoretically pure mycelial lectin (PCL-M) mixed with an equal volume of complete Freund's adjuvant. Preparation of anti-PCL-F serum and the immunoblotting proceeded as described elsewhere (8, 24).

Preparation of peptides. PCL-M was S-pyridylethylated after reduction by dithiothreitol (3), dissolved in 20 mM Tris-HCl (pH 9.0) containing 4 M urea, and incubated with lysylendopeptidase (Wako Pure Chemicals) at 30°C for 20 h. Peptides were purified by reverse-phase high-performance liquid chromatography (HPLC) on a column of TSK Phenyl 5PW RP (4.6 mm by 7.5 cm) (Tosoh) with a gradient of acetonitrile in 0.1% trifluoroacetic acid.

TABLE 1. Purification of PCL-M

	Protein (mg)	Titer (10^3)	Sp act (titer [10^2]/mg)	Yield (%)
Crude extract	2,130	29.7	0.14	100
Ammonium sulfate	984	29.7	0.30	100
PSM-Sepharose 4B	0.75	14.8	198	50

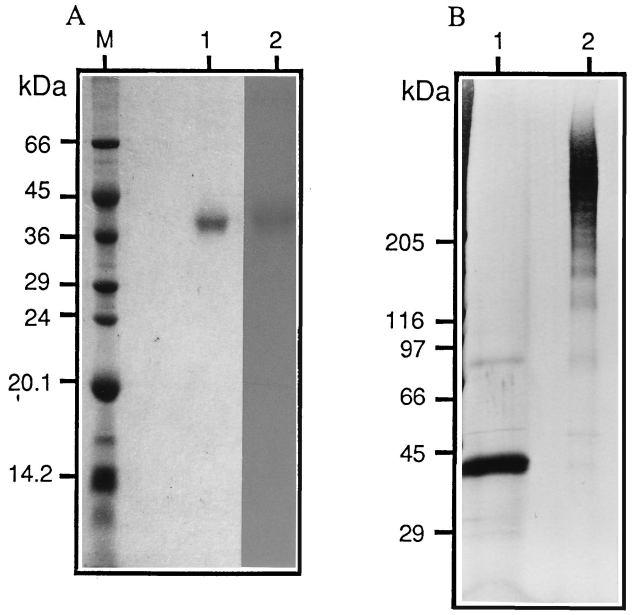


FIG. 3. SDS-PAGE of PCL-M. (A) Purified PCL-M (2 μg) was resolved by SDS-PAGE using a 12.5% gel and stained by Coomassie brilliant blue (lane 1) or by the periodic acid-Schiff reagent (lane 2). (B) Purified PCL-M (2 μg) was resolved by SDS-PAGE using a 4 to 15% gradient gel in the presence (lane 1) or absence (lane 2) of 2-ME.

Analytical methods. Amino acids were identified in a Hitachi model 835 analyzer. Samples (80 μg) were hydrolyzed in 6 M HCl containing 0.01% phenol in sealed evacuated tubes for 24 h at 110°C. Cysteine was measured as cysteic acid after performic acid oxidation. Tryptophan was measured spectrophotometrically (6). Automated sequencing proceeded in a pulsed liquid-phase sequencer (Applied Biosystems model 477A/120A). The N-terminal amino acid sequence was analyzed with S-pyridylethylated PCL-M.

RESULTS

Appearance of hemagglutinating activity at four growth phases. Hemagglutinating activities in crude extracts prepared from the two strains, KC-1 and KC-2, of *P. cornucopiae* at various growth phases were examined. For convenience, growth phases of this organism were divided into four stages in relation to their developmental process: stage 0, mycelia grown in liquid medium; stage I, mycelia grown on solid medium; stage II, primordia or immature fruit bodies; and

TABLE 2. Effects of divalent cations and 2-ME on PCL-M activity[a]

1st addition[b]	2nd addition[c]	Titer
Expt 1		
None	$CaCl_2$ + EDTA	320
EDTA	None	0
EDTA	$CaCl_2$	320
EDTA	$MnCl_2$	25
EDTA	$MgCl_2$	8
Expt 2		
None		128
2-ME		0

[a] PCL-M was dissolved in TBS containing 5 mM $CaCl_2$.
[b] Followed by a 1-h incubation at 4°C. Final concentrations: EDTA, 20 mM; 2-ME, 2 mM.
[c] Followed by a 12-h incubation. Final concentration, 100 mM.

TABLE 3. Carbohydrate-binding specificities of PCL-M

Inhibitor	Concn[a]
N-Acetyl-D-galactosamine	4 mM
N-Acetyl-D-galactosamine tetramer	4 mM
D-Fucose	30 mM
D-Galactose	40 mM
D-Galactosamine	50 mM
Lactose	20 mM
Lactosamine	20 mM
Melibiose	26 mM
Raffinose	50 mM
BSM	16.5 μg/ml
Asialo-BSM	0.3 μg/ml
PSM	0.4 μg/ml
Fetuin	>1,350 μg/ml
Asialo-fetuin	>1,100 μg/ml
Thyroglobulin	610 μg/ml

[a] Minimum concentration for complete inhibition of hemagglutination.

stage III, mature fruit bodies. As shown in Fig. 1, no hemagglutinating activity was found in the extracts of the stage 0 mycelia, whereas activity was measurable in the stage I mycelia of both strains. Since strain KC-1 produces PCL-F (8), the activity increased in stages II and III, but in strain KC-2 it decreased. As described below, the active substance found in the stage I mycelia was not PCL-F; it was named PCL-M (mycelial lectin of *P. cornucopiae*) to distinguish it from PCL-F.

Purification. PCL-M was purified by elution through a PSM-Sepharose 4B affinity column with EDTA (Fig. 2). The hemagglutinating activity of PCL-M was assayed after the addition of $CaCl_2$. A summary of the purification is shown in Table 1. PCL-M was purified to homogeneity and over 1,400-fold by this procedure.

Molecular mass and subunit construction. As shown in Fig. 3A, PCL-M migrated as a single band with a molecular mass of 40 kDa in SDS-PAGE under reducing conditions, and it was a glycoprotein, since it was stained by the periodic acid-Schiff reagent on the gel. In the absence of 2-ME, PCL-M formed multimers of the 40-kDa subunit, showing ladder-like bands with apparent molecular masses ranging from 80 to 600 kDa (Fig. 3B).

Effects of EDTA and 2-ME. As shown in Table 2, the hemagglutinating activity of PCL-M was inhibited by EDTA. The activity was fully restored by $CaCl_2$, whereas $MnCl_2$ or $MgCl_2$ was less effective. The activity of PCL-M was also abolished completely by 2-ME, indicating that the polymerized form of PCL-M is active.

Specificity for sugar binding. Table 3 shows that *N*-acetyl-D-galactosamine and its tetramer had the same inhibitory effect on the hemagglutinating activity of PCL-M.

TABLE 4. Amino acid composition of PCL-M and PCL-F

Residue	Mol[a]	
	PCL-M (362.9)	PCL-F[b] (141.9)
Asx	37.4	17.2
Thr	28.7	10.2
Ser	39.2	9.4
Glx	35.2	14.5
Pro	18.9	3.2
Gly	52.2	14.4
Ala	37.0	12.9
Cys[c]	4.4	0
Val	26.5	10.9
Met	0.7	3.7
Ile	15.1	6.9
Leu	14.2	9.4
Tyr	6.1	4.8
Phe	12.2	5.7
Lys	13.3	5.9
His	6.2	2.2
Arg	8.6	9.6
Trp[d]	6.8	1.0

[a] Totals are given in parentheses.
[b] From reference 24.
[c] Measured as cysteic acid.
[d] Measured spectrophotometrically (6).

TABLE 5. Partial amino acid sequences of PCL-M

Lectin	Sequence[a]
N terminus	1 10 19
PCL-M	TNPTRKGKMVPSAFRPAFL
PCL-F[b]	Ac-SGSGTSGGMLRFT
Internal	194 201
Discoidin I[c]	TLPKVAL--NFDQIDCTDA
	+++ ++ ++
PCL-M[d]	TLPGVAPPVNFVTSAFD

[a] +, identical amino acid residues; dashes, gaps inserted to maximize the match.
[b] From reference 15.
[c] From reference 16.
[d] Peptide KM-24.

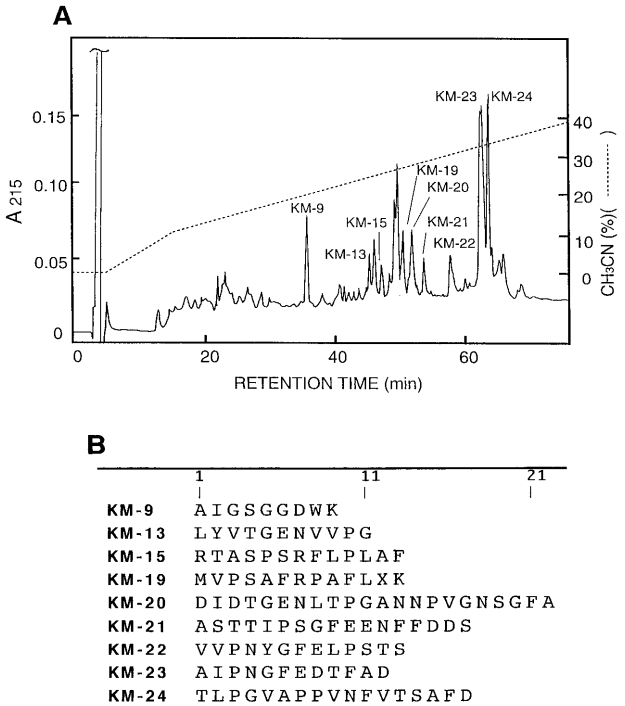


FIG. 4. Isolation and sequence determination of PCL-M peptides. (A) A lysylendopeptidase digest of PCL-M was applied to a column of Phenyl 5PW-RP (4.6 mm by 7.5 cm) (Tosoh), and peptides were eluted by a gradient of acetonitrile in 0.1% trifluoroacetic acid. (B) Sequences of nine isolated peptides.

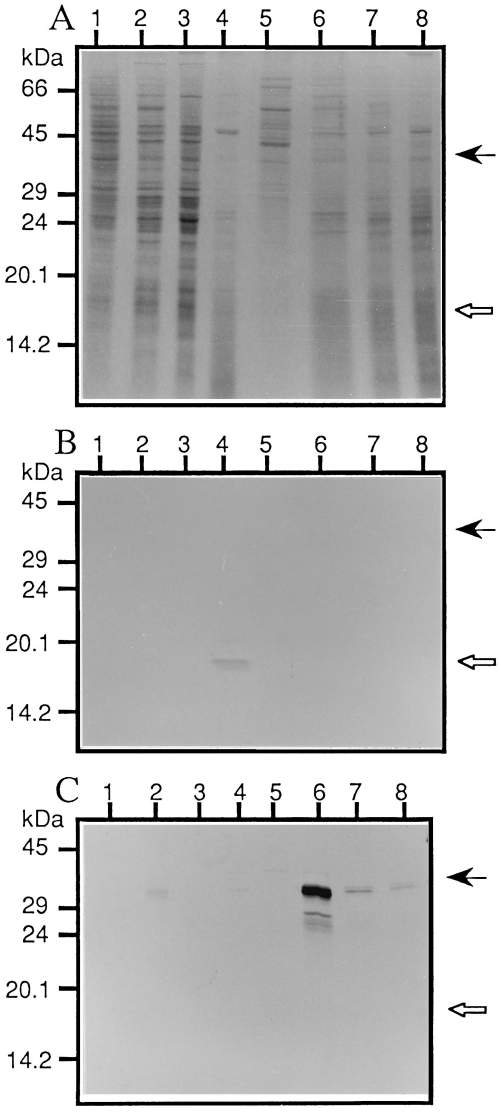
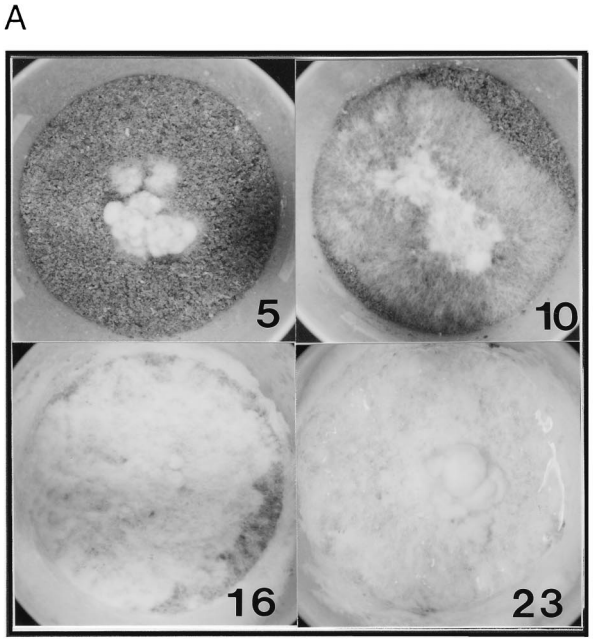


FIG. 5. Stage-specific expression of PCL-F and PCL-M. Crude extracts (20 μg of protein equivalent) prepared from *P. cornucopiae* at each of the four growth phases as described in the legend to Fig. 1 were resolved by SDS–12.5% PAGE. Protein bands were visualized by staining with Coomassie brilliant blue (A). After the protein bands were electroblotted onto nitrocellulose paper, PCL-F (B) or PCL-M (C) was visualized with the respective antiserum. The positions of PCL-F and PCL-M are indicated by open and filled arrows, respectively. Lanes 1 to 4, extracts from strain KC-1; lanes 5 to 8, extracts from strain KC-2. Lanes 1 and 5, stage 0 mycelia; lanes 2 and 6, stage I mycelia; lanes 3 and 7, stage II fruit bodies; lanes 4 and 8, stage III fruit bodies.

The saccharides having a galactosyl moiety inhibited the activity to some extent. The following saccharides did not inhibit the activity up to 200 mM: L-fucose, D-fructose, D-ribose, D-xylose, D-mannose, D-glucosamine, *N*-acetyl-D-glucosamine, maltose, and sucrose. The activity was also inhibited by BSM and PSM but not by fetuin and thyroglobulin. The inhibitory effect of BSM was increased by the removal of sialic acids. PCL-M agglutinated human type A, B, and O erythrocytes equally. Rabbit erythrocytes were agglutinated more efficiently than human erythrocytes.

Amino acid composition, sequencing, and homology. The amino acid composition of PCL-M is shown in Table 4. PCL-M was relatively rich in hydrophobic amino acids and contained 4 mol of half-Cys as contrasted with PCL-F. The N-terminal amino acid sequences of PCL-M and PCL-F are shown in Table 5. The sequences were different from each other. The N-terminal sequence of PCL-M showed no significant homology with those in the Swiss-Prot database. As shown in Fig. 4, nine internal peptides were obtained by digesting PCL-M with lysylendopeptidase and then separating by reverse-phase HPLC. Partial amino acid sequences of the peptides were determined; their sum accounted for approximately one-third of the total residues of PCL-M. The sequence of one peptide was partly homologous with that of discoidin I, a lectin produced by *Dictyostelium discoideum* (16) as shown in Table 5.

Developmental stage-specific expression. Crude extracts prepared from the two strains of *P. cornucopiae* at four developmental stages were resolved by SDS-PAGE and then immu-



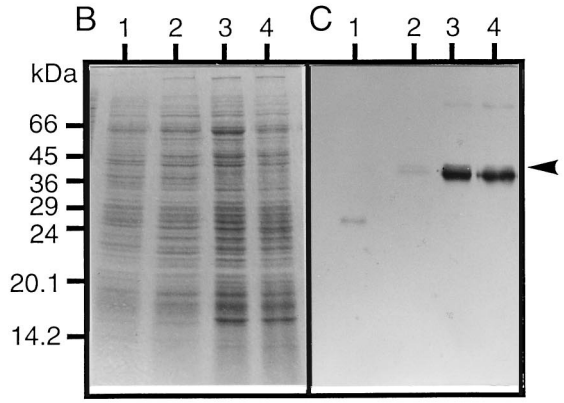


FIG. 6. Expression of PCL-M during stage I. (A) Growth of mycelia of strain KC-2 at days 5, 10, 16, and 23 on solid medium. Crude extracts (20 μg of protein equivalent) prepared from each mycelium grown for 5 (lane 1), 10 (lane 2), 16 (lane 3), or 23 (lane 4) days were resolved by SDS–12.5% PAGE and immunoblotted. Protein bands (B) and PCL-M (C) were stained as described in the legend to Fig. 5.

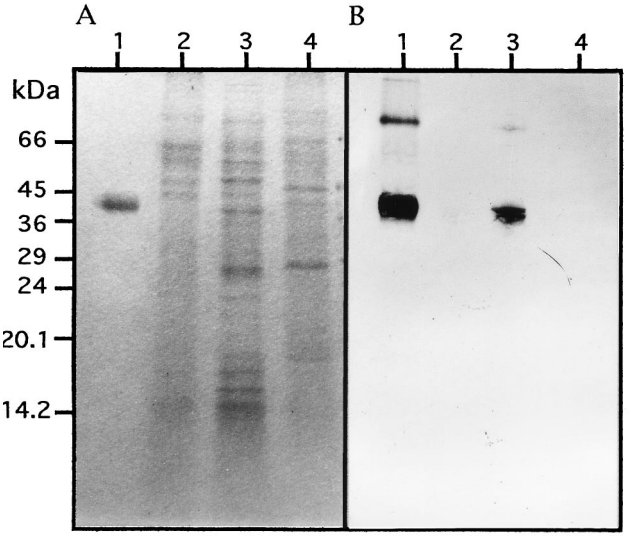


FIG. 7. Dikaryotic but not monokaryotic mycelia produce PCL-M. Dikaryotic and monokaryotic mycelia derived from *P. cornucopiae* KC-2 were grown on solid medium. Crude extracts (20 μg of protein equivalent) were prepared, resolved by SDS–12.5% PAGE, and immunoblotted. Protein bands (A) and PCL-M (B) were stained as described in the legend to Fig. 5. Lane 1, purified PCL-M; lanes 2 and 4, monokaryotic mycelia having a different mating type; lane 3, dikaryotic mycelia.

noblotted against anti-PCL-F or anti-PCL-M serum. As shown in Fig. 5B, when anti-PCL-F serum was used, the stage III fruit bodies of strain KC-1 gave a 16-kDa band of PCL-F, whereas no band was found in the stage I mycelia of either strain, although these samples showed potent hemagglutinating activities (Fig. 1). Figure 5C shows that the stage I mycelia of both strains reacted with anti-PCL-M serum, giving a 40-kDa band of PCL-M, although the signal in strain KC-1 was somewhat weak. The band intensity of PCL-M in strain KC-2 was very high in stage I and weak in stages II and III. These results indicate that (i) PCL-M is immunochemically different from PCL-F, (ii) strain KC-2 does not produce PCL-F but does produce PCL-M, (iii) PCL-M is synthesized preferentially in the stage I mycelia, and (iv) PCL-M disappears during stages II and III.

As the period of stage I was 20 days, we examined the time course of PCL-M appearance. Growth of mycelia on the solid medium at days 5, 10, 16, and 23 and the results of immunoblotting after SDS-PAGE are presented in Fig. 6. PCL-M was expressed only after the 16th day of culture, when mycelia had proliferated all over the medium and primordia might have formed.

Dikaryotic but not monokaryotic mycelia produced PCL-M. In basidiomycetes, dikaryotic but not monokaryotic mycelia form fruit bodies. Since the dikaryotic mycelia synthesized PCL-M preferentially at stage I (Fig. 5), we examined whether monokaryotic mycelia at stage I produced PCL-M. Several monokaryons were obtained by single-spore isolation from the fruit body of strain KC-2, and the mating types were determined by the confronting culture. Two monokaryons with different mating types were selected, and a dikaryon was obtained by mating the two monokaryons. They were grown on the solid medium, and the extracts were resolved by SDS-PAGE and immunoblotted. As shown in Fig. 7, although the dikaryon gave a band reactable with anti-PCL-M serum, no band was detected in the extracts from the two monokaryons.

Surface localization. To examine the localization of PCL-M, a piece of mycelium was mixed with rabbit erythrocytes and examined under the microscope. As shown in Fig. 8, erythrocytes formed aggregates on the surface of stage I mycelia but not stage 0 mycelia. Adhesion of erythrocytes to mycelia occurred within 1 min, and this was inhibited by the addition of asialo-BSM, EDTA, or anti-PCL-M serum, the inhibitors of hemagglutination by PCL-M.

DISCUSSION

Lectins have been found and purified from fruit bodies, but very few have been identified in vegetative mycelia. We found that *P. cornucopiae* KC-1 synthesized two lectins, PCL-F and PCL-M, in the developmental stage-specific manner: PCL-F was synthesized in the fruit body (8), whereas PCL-M was synthesized only in the solid-medium-grown mycelia. The two lectins were different from biochemical and immunochemical points of view (14, 15, 24). A mycelial lectin from the nematode-trapping fungus *Arthrobotrys oligospora* (17) has been reported as a surface lectin. However, unlike PCL-M this lectin contained no sulfur amino acids, and the sugar-binding specificities of the two lectins were different.

The degrees to which wheat germ agglutinin is inhibited by GlcNAc and by a trimer of GlcNAc widely differ because of the structural features of the binding site (12, 21). In contrast, GalNAc and its tetramer showed the same inhibitory effect upon PCL-M, suggesting that its sugar-binding site does not have subsites.

PCL-M lost the activity upon depletion of Ca^{2+} by EDTA. Ca^{2+} is required by animal C-type lectins (5) and discoidin I (1). In the amino acid sequences of PCL-M peptides so far examined, some similarity with that of discoidin I was found; however, no significant homology with those of animal C-type lectins was found, although some of them had similar carbohydrate specificity.

In basidiomycetes under specific physiological and environmental conditions, dikaryotic mycelia begin to form fruit bodies. Fruit body formation is accompanied by the transcription of a small number of specific genes that produce abundant mRNAs. One such gene product in *Schizophyllum commune* (22) or in *Agaricus bisporus* (4) is a group of hydrophobic proteins referred to as "hydrophobins." In *Lentinus edodes* the product of such a gene, *priB*c, has been identified (7). We examined the occurrence of lectins at the three developmental stages in *P. cornucopiae* to clarify physiological functions of lectins in nature. The results presented here demonstrated that PCL-M is synthesized only in mycelia that are ready to form the fruit body, and it is localized on the surface of such mycelia. PCL-M should be essential for fruiting, since all the strains of *P. cornucopiae* so far examined (eight commercially available and eight wild-type strains) contained this lectin as determined by hemagglutinating activity and immunoblotting, although the content varied among strains.

On the basis of these results it is suggested that PCL-M participates in some way during the process of fruit body formation in *P. cornucopiae*. Cell walls of fungi play essential roles in growth and many other functions including morphological responses, antigenic expression, adhesion, and cell-cell interaction (20). PCL-M may stimulate the formation of primordia in *P. cornucopiae* by adhering hyphae to each other, although the existence of *N*-acetylgalactosamine in its cell wall is still unknown.

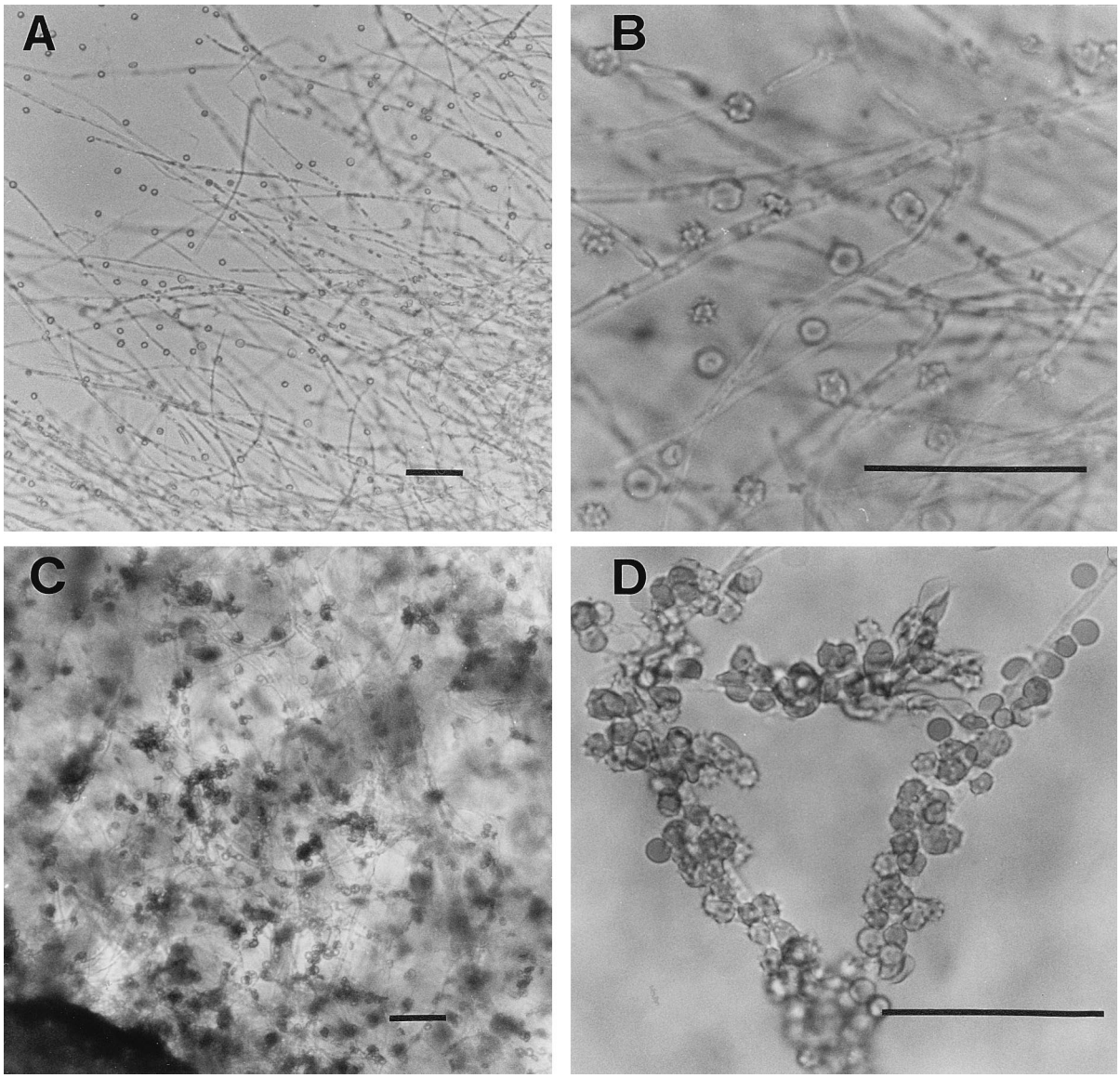


FIG. 8. Localization of PCL-M. Mycelia grown in liquid medium (A and B) or on solid medium (C and D) were mixed with a 1% suspension of rabbit erythrocytes. After a 5-min incubation, the samples were examined under the microscope at magnifications of ×100 (A and C) and ×400 (B and D). Bars, 0.1 mm.

REFERENCES

1. **Alexander, S., A. M. Cibulsky, and R. A. Lerner.** 1983. Ion dependence of the disoidin I lectin from *Dictyosterium discoideum*. Differentiation **24:**209–212.
2. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
3. **Charbonneau, H.** 1989. Strategies for obtaining partial amino acid sequence data from small quantities of pure or partial purified protein, p. 17–32. *In* P. T. Matsudaira (ed.), A practical guide to protein and peptide purification for microsequencing. Academic Press, San Diego, Calif.
4. **De Groot, P. W. J., P. J. Schaap, A. S. M. Sonnenberg, J. Visser, and L. J. L. D. Van Griensven.** 1996. The *Agaricus bisporus hyp*A gene encodes a hydrophobin and specifically accumulates in peel tissue of mushroom caps during fruit body development. J. Mol. Biol. **257:**1008–1018.
5. **Drickamer, K.** 1988. Two distinct classes of carbohydrate-recognition domains in animal lectins. J. Biol. Chem. **263:**9557–9560.
6. **Edelhoch, H.** 1967. Spectroscopic determination of tryptophan and tyrosine in proteins. Biochemistry **6:**1948–1954.
7. **Endo, H., S. Kajiwara, O. Tsunoka, and K. Shishido.** 1994. A novel cDNA, *priB*c, encoding a protein with a Zn(11)2Cys6 zinc cluster DNA-binding motif, derived from the basidiomycete *Lentinus edodes*. Gene **139:**117–121.
8. **Kaneko, T., S. Oguri, S. Kato, and Y. Nagata.** 1993. Developmental appearance of lectin during fruit body formation in *Pleurotus cornucopiae*. J. Gen. Appl. Microbiol. **39:**93–100.
9. **Kawagishi, H., A. Nomura, T. Mizuno, A. Kiyama, and S. Chiba.** 1990. Isolation and characterization of a lectin from *Grifola fondosa* fruiting bodies. Biochim. Biophys. Acta **1034:**247–252.
10. **Kochibe, N., and K. L. Matta.** 1989. Purification and properties of an N-acetylglucosamine-specific lectin from *Psathyrella velutina* mushroom. J. Biol. Chem. **264:**173–177.
11. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
12. **Nagata, Y., and M. M. Burger.** 1973. Wheat germ agglutinin: molecular characteristics and specificity for sugar binding. J. Biol. Chem. **249:**3116–3122.
13. **Nagata, Y., F. Fukumori, H. Sakai, T. Hagiwara, Y. Hiratsuka, N. Kochibe, and A. Kobata.** 1991. Crystallization and characterization of a lectin obtained from a mushroom, *Aleuria aurantia*. Biochim. Biophys. Acta **1076:**187–190.
14. **Oguri, S., and Y. Nagata.** 1994. Complete amino acid sequence of a lectin-related 16.5 kDa protein isolated from fruit bodies of lectin-deficient strain of *Pleurotus cornucopiae*. Biosci. Biotechnol. Biochem. **58:**507–511.
15. **Oguri, S., M. Yoshida, and Y. Nagata.** 1994. Isolation, crystallization and characterization of a 16.5kDa protein from fruit bodies of lectin-deficient strain of *Pleurotus cornucopiae*. Biosci. Biotechnol. Biochem. **58:**502–506.

16. **Poole, S., R. A. Firtel, and E. Lamar.** 1981. Sequence and expression of the discoidin I family in *Dictyostelium discoideum*. J. Mol. Biol. **153:**273–289.
17. **Rosen, S., B. Ek, L. Rask, and A. Tunlid.** 1992. Purification and characterization of a surface lectin from the nematode-trapping fungus *Arthrobotrys oligospora*. J. Gen. Microbiol. **138:**2663–2672.
18. **Sage, H. J., and S. L. Connett.** 1969. Studies on a hemagglutinin from the meadow mushroom. J. Biol. Chem. **244:**4713–4719.
19. **Segrest, J. P., and R. L. Jackson.** 1972. Molecular weight determination of glycoproteins by polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Methods Enzymol. **28:**54–63.
20. **Sentandreu, R., S. Mormeneo, and J. Ruiz-Herrera.** 1994. Biogenesis of the fungal cell wall, p. 111–124. *In* K. Esser and P. A. Lemke (ed.), The mycota, vol. I. Springer-Verlag, Berlin.
21. **Sharon, N., and H. Lis.** 1989. Lectins, p. 20–25. Chapman and Hall, New York.
22. **Wessels, J. G. H.** 1992. Gene expression during fruiting in *Schizophyllum commune*. Mycol. Res. **96:**609–620.
23. **Yatohgo, T., Y. Nakata, Y. Tumuraya, Y. Hashimoto, and Y. Yamamoto.** 1988. Purification and properties of a lectin from the fruitbodies of *Flammulia velutipes*. Agric. Biol. Chem. **52:**1485–1493.
24. **Yoshida, M., S. Kato, S. Oguri, and Y. Nagata.** 1994. Purification and properties of lectins from a mushroom *Pleurotus cornucopiae*. Biosci. Biotechnol. Biochem. **58:**498–501.