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Hemagglutinins (lectins) in fruit bodies of Japanese higher fungi

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Extracts from fruit bodies of 110 species of Japanese fungi were examined with trypsinized human and rabbit erythrocytes. More than 80% of the extracts showed the hemagglutination activities, a higher proportion than reported previously. Over half of species that had been reported to be inactive exhibited hemagglutination. Among them, some extracts showed human blood group specific-hemagglutination; A-specific, *Panellus serotinus, Psathyrella piluliformis, Cantharellus cibarius* and *Stropharia rugosoannulata*; B-, O-specific, *Gyroporus castaneus* and *Panellus stypticus*; and O-specific, *Linderia bicolumnata* and *Phallus impudicus*. Twenty-one species were reactive toward only rabbit erythrocytes. Several species exhibited very high hemagglutination activity. The results suggested that some of these Japanese fungi would be promising sources of lectins.

Key Words——fungi; hemagglutinin; lectin; mushroom.

Many lectins are now commercially available because of their usefulness in biological and medical fields. They recognize carbohydrate structures on cell surfaces and have been used for blood typing. However, the recent accumulation of knowledge of carbohydrate structures in animal, plant and microbial cells points to the needs for more lectins with higher specificity or stronger affinity toward carbohydrate structures. Of fungal lectins, those of *Agaricus bisporus* (J. Lange) Imbach (Presant and Kornfeld, 1972; Sueyoshi et al., 1985) and *Aleuria aurantia* (Fr.) Fuckel (Kochibe and Furukawa, 1980) are now commercially available. These and many other lectins have been purified from fruit bodies of Japanese fungi (Kawagishi et al., 1988, 1990, 1991, 1994, 1996, 1997; Kawagishi and Mizuno, 1988; Kochibe and Matta, 1989; Tsuda, 1979; Yagi et al., 1997; Yagi and Tadera, 1988; Yatohgo et al., 1988; Yoshida et al., 1994; Zhuang et al., 1996). Some of the fungal lectins exhibit unique carbohydrate-binding specificities. *Agrocybe cylindracea* (DC.: Fr.) Maire lectin recognizes trisaccharide structures with terminal α2,3-linked *N*-acetylneuraminic acid (Yagi et al., 1997). *Hericium* lectin recognizes *N*-glycolylneuraminic acid (Kawagishi et al., 1994). *Psathyrella veltina* (Pers.) Sing. lectin has affinity for *N*-acetyl glucosamine and *N*-acetylneuraminic acid (Kochibe and Matta, 1989; Ueda et al., 1999). These results mean that fruit bodies of fungi are potential sources of lectins.

Several reports have appeared on the hemagglutination activity of fungi, although discrepancies are also found among them. In this respect, Pemberton (1994) showed that the specificity and activity of some species vary with location. Also lectins have been purified from fruit bodies of *Grifola frondosa* S. F. Gray (Kawagishi et al., 1990), *Ganoderma lucidum* (Leyss.: Fr.) Karst. (Kawagishi et al., 1997), and *Pleurotus cornucopiae* (Paillet) Rolland var. *citrinopileatus* (Sing.) Ohira (Yoshida et al., 1994), though hemagglutinating activity was not detected in other extracts of these mushrooms (Pemberton, 1994; Merland and Coulet, 1961; Coulet et al., 1964). Furthermore, in some studies, only a less sensitive assay with native human erythrocytes was used for hemagglutination. These results suggested the need to test for hemagglutination activity in fruit bodies of Japanese fungi, even if some of the species had already been tested in other countries.

For Japanese higher fungi, Furukawa et al.(1995) reported that they had surveyed 833 species of fungi with human erythrocytes, but their report described only 8 species which were human blood type-specific. The distribution of hemagglutination activity in Japanese fungi remains largely unknown.

This report deals with a survey of hemagglutination activity in Japanese higher fungi mainly collected in southern Kyushu. Trypsinized human and rabbit erythrocytes were used to test hemagglutination with higher sensitivity, because protease-treated erythrocytes are known to agglutinate more easily than native ones.

Materials and Methods

Materials Fruit bodies of fungi were collected mainly in Kumamoto and Kagoshima prefectures, southern Kyushu, in 1994–1999. Fresh fruit bodies were kept at −20°C before analysis or lyophilized as soon as possible after collection. Fresh or frozen samples were homogenized with 10 volumes of phosphate-buffered saline (PBS), pH 7.2, that contained 6.7 mM phosphate (K,

Table 1. Source and activity type of fungi.

BASIDIOMYCOTINA [EUBASIDIOMYCETES] HYMENOMYCETIDAE		Collecting place	Activity type	Survey results
Agaricales				
1	*Agaricus abruptibulbus* Peck	3		
2	*Agrocybe cylindracea* (DC.: Fr.) Maire	C		
3b	*Amanita castanopsidis* Hongo	2	NO	
4c	*A. pseudoporphyria* Hongo	3		
5ab	*A. rubescens* Pers.: Fr.	3	H, NO	
6c	*A. spissacea* Imai	7		
7ab	*A. vaginata* (Bull.: Fr.) Vitt. var. *vaginata*	1	NO	
8c	*A. virgineoides* Bas	3	NO	
9ab	*Armillariella mellea* (Vahl.: Fr.) Karst.	5	NO	
10b	*Chlorophyllum molybdites* (Meyer: Fr.) Massee	1		
11a	*Clitocybe clavipes* (Pers.: Fr.) Kummer	9		
12a	*C. gigga* (Pers.: Fr.) Kummer	3		
13a	*Collybia confluens* (Pers.: Fr.) Kummer	9	R	
14a	*C. dryophila* (Bull.: Fr.) Kummer	6		
15a	*Coprinus micaceus* (Bull.: Fr.) Fr.	3		
16	*Cortinarius salor* Fr.	9		
17	*Crinipellis stipitaria* (Fr.) Pat.	1		
18c	*Descolea flavoannulata* (L. Vassil.) Horak	9		
19c	*Galerina fasciculata* Hongo	3	R	
20b	*Gomphidius roseus* (Fr.) Karst.	4	NO	
21	*Gymnopilus liquiritiae* (Pers.: Fr.) Karst.	6		
22a	*Gyroporus castaneus* (Bull.: Fr.) Quél.	6	B-O	
23a	*Hygrocybe coccinea* (Schaeff.: Fr.) Kummer	10		
24	*H. flavescens* (Kauffm.) Sing.	3		
25	*Hypsizigus marmoreus* (Peck) Bigelow	C		
26c	*Lactarius laeticolorus* (Imai) Imaz.	4		
27a	*L. quietus* Fr.	9		Same
28	*L. subvellereus* Peck	6		
29c	*L. subzonarius* Hongo	3		
30c	*Laccaria vinaceoavellanea* Hongo	3	NO	
31c	*Lampteromyces japonicus* (Kawam.) Sing.	6	H, NO	
32c	*Lyophyllum sykosporum* Hongo et Clémençon	6		
33a	*Macrolepiota procera* (Scop.: Fr.) Sing.	3	NO	
34c	*Marasmius maximus* Hongo	9	R	
35	*Mycena haematopoda* (Pers.: Fr.) Kummer	7		
36a	*Naematoloma fasciculare* (Hudson: Fr.) Karst.	7		
37ab	*Oudemansiella mucida* (Schrad: Fr.) Höhnel	5	R	
38a	*O. radicata* (Relhan: Fr.) Sing.	7		Same
39a	*Panellus serotinus* (Pers.: Fr.) Kühn	6	A	
40a	*P. stypticus* (Bull.: Fr.) Karst.	5	B-O	
41b	*Panus tigrinus* (Bull.: Fr.) Sing.	1		
42bc	*Phylloporus bellus* (Mass.) Corner	1		
43c	*Pleurotus abalonus* Han & al.	C		
44bc	*P. salmoneostramineus* L. Vass.	C		
45a	*Psathyrella candolleana* (Fr.: Fr.) Maire	7		
46a	*P. piluliformis* (Bull.: Fr.) P.D. Orton	7	A	
47c	*Rhodophyllus ater* Hongo	3		
48	*R. clypeatus* (L.) Quél.	8		
49a	*Rozites caperata* (Pers.:Fr.) Karst.	9		
50a	*Russula emetica* (Schaeff.: Fr.) S. F. Gray	4	NO	Same

Table 1. continued

51	*R. flavida* Frost et Peck apud Peck	6		
52ab	*R. laurocerasi* Melzer	4		Same
53a	*R. nigricans* (Bull.) Fr.	4		
54	*R. rosacea* (Pers.) S. F. Gray	3		
55b	*R. violeipes* Quél.	3		
56a	*Schizophyllum commune* Fr.: Fr.	1	R	
57b	*Strobilomyces confusus* Sing.	1	NO	
58	*Stropharia rugosoannulata* Farlow	3	A	
59a	*Suillus bovinus* (L.: Fr.) O. Kuntze	4	R	
60ab	*S. granulatus* (L.: Fr.) O. Kuntze	8		
61a	*S. luteus* (L.:Fr.) S. F. Gray	5	NO	Same
62b	*Tricholoma giganteum* Massee	2	R	
63ab	*T. saponaceum* (Fr.) Kummer	8	R	
64ab	*T. ustale* (Fr.: Fr.) Kummer	4		Same
65a	*Tricholomopsis rutilans* (Schaeff.: Fr.) Kummer	7		Same
66	*Tylopillus ballouii* (Peck) Sing.	7	NO	
67c	*T. valens* (Corner) Hongo et Nagasawa	3	R	
68c	*T. virens* (Chiu) Hongo	2		
Aphyllophorales				
69a	*Cantharellus cibarius* Fr.	7	A	
70	*Clavicorona pyxidata* (Fr.) Doty	8		
71	*Coltricia cinnamomea* (Pers.) Murr.	3		
72	*Coriolus hirsutus* (Wulf.: Fr.) Quél.	5	R	
73c	*Cryptoporus volvatus* (Peck) Shear	4	R	
74c	*Daedalea dickinsii* (Berk. ex Cooke) Yasuda	5	R	
75c	*Daedaleopsis purpurea* (Cooke) Imaz. et Aoshi.	5		
76	*D. tricolor* (Bull.: Fr.) Bond. et Sing.	6	R	
77	*Elfvingia applanata* (Pers.) Karst	6	R	
78ab	*Hydonum repandum* L.: Fr. var. *album* Quél.	3		
79	*Microporos flabelliformis* (Fr.) Kuntze	3		
80	*M. vernicipes* (Berk.) Kuntze			
81b	*Perenniporia fraxinea* (Fr.) Ryv.	1		
82	*P. ochroleuca* (Berk.) Pilat	3		
83b	*Polyporus arcularius* Batsch.: Fr.	3		
84a	*P. brumalis* (Fr.) Karst.	6		
85bc	*P. tenuiculus* (Beauv.) Fr.	1	H, R	
86a	*P. varius* (Pers.: Fr.) Karst.	7		
87	*Pycnoporus coccineus* (Fr.) Bond et Sing.	6		
88	*Ramariopsis kunzei* (Fr.) Donk	2		
89a	*Stereum gausapatum* Fr.: Fr.	2	R	
90c	*Thelephora aurantiotincta* Corner	10		
91	*Trichaptum elongatum* (Berk.) Imaz.	3	NO	
92a	*T. fuscoviolaceum* (Dicks.: Fr.) Ryv.	3	R	
93c	*Tyromyces incarnatus* Imaz.	3		
94bc	*Xylobolus princeps* (Jungh.) Boiden	3		
GASTEROMYCETIDAE				
Sclerodermatales				
95	*Astraeus hygrometricus* (Pers.) Morgan	3	NO	
96	*Pisolithus tinctorius* (Pers.) Coker et Couch	3		
Nidulariales				
97b	*Cyathus stercoreus* (Schw.) De Toni	3		
Lycoperdales				
98	*Calvatia caelata* (Bull.) Morgan	10		

Table 1. continued

99	*C. craniiformis* (Schw.) Fr.	4	R	
100a	*Lycoperdon perlatum* Pers.: Pers.	3	R	
Phallales				
101b	*Linderia bicolumnata* (Lloyd) Cunn.	3	O	
102a	*Phallus impudicus* L.: Pers.	3	O	Same
[HETEROBASIDIOMYCETES]				
Tremellales				
103a	*Tremella foliacea* Pers.: Fr.	3	NO	Same
Auriculariales				
104	*Auricularia polytricha* (Mont.) Sacc.	C		
Dacrymycetales				
105	*Dacrymyces palmatus* (Schw.) Burt.	3	R	
ASCOMYCOTINA				
[DISCOMYCETES]				
Helotiales				
106	*Ascoclavulina sakaii* Otani	9		
107	*Ascocoryne cylichnium* (Tul.) Korf	9		
108a	*Leotia lubrica* (Scop.) Pers.: Fr. f. *lubrica*	3		
Pezizales				
109	*Peziza praetervisa* Bres.	3		
[PYRENOMYCETES]				
Xylariales				
110a	*Xylaria polymorpha* (Pers.) Grev.	3	R	

a, previously investigated.
b, lyophilized sample was used.
c, Species found only in Japan or Japan and Asia.
Collecting place: 1, Kagoshima University Korimoto campus; 2, Kagoshima city; 3, Ijuin-cho; 4, Fukiage-cho; 5, Mt. Kirishima; 6, Kikuchi Valley; 7, Mt. Shotai; 8, Hitoyoshi city; 9, Mt.Kuju; 10, other places; C, commercially obtained.
A, B-O, O were specific toward A-type, B and O-type, and O-type erythrocytes. R, only active toward rabbit erythrocytes. H, hemolytic activity. NO, no hemagglutination activity.
Same, no contradiction with results previously reported.

Na), 132 mM NaCl and 0.04% NaN_3. Homogenates were passed through two layers of gauze, then centrifuged at 7,000 × *g* for 20 min to yield clear supernatants. Lyophilyzed samples or woody samples were powdered and extracted with 30–100 volumes of PBS for several hours at 4°C.

Hemagglutination assay Hemagglutinating activity was determined in wells of microtiter plates by the 2-fold dilution method, in a final volume of 70 μl of PBS. Each well contained 50 μl of lectin solution and 20 μl of a 4% (v/v) suspension of trypsinized human or rabbit erythrocytes.

Agglutination was assessed after incubation for 1 h at room temperature, and hemagglutinating activity was expressed as the titer, namely, the reciprocal of the highest dilution that gave a positive result. The specific hemagglutinating activity was defined as the titer/mg protein or the titer/g fresh weight. If the hemagglutination was observed over 1024 titer, the 2-fold dilution was started with the dilution of crude extract to 1/1024.

When hemagglutination activity was not detected in the crude extracts, protein in the extract was precipitated with 100% saturation of $(NH_4)_2SO_4$ and the concentrated solution (at least 1 mg protein/ml PBS) was used for hemagglutination assay.

When a difference of over 8-fold hemagglutination was observed between one human blood type and other types, the hemagglutination was defined as being blood group-specific.

Quantitation of protein Protein was quantitated by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Results and Discussion

Ratio of active species Table 1 lists the fungal species studied, and Table 2 summarizes their hemagglutination activity. In a few instances, extracts contained strong hemolytic activity: notable were *Amanita rubescens* Pers.: Fr. (5), *Lampteromyces japonicus* (Kawam.) Sing. (31). The extract of *Polyporus tenuiculus* (Beauv.) Fr. (85) showed hemagglutination of rabbit erythrocytes but hemolysis of human erythrocytes. Sixteen species (3, 5, 7–9, 20, 30, 31, 33, 50, 57, 61, 66, 91, 95, 103) exhibited no hemagglutination for human ABO and rabbit erythrocytes, whereas 94 species exhibited hemagglutination. The proportion of active species (94/110, 85%)

Table 2. Hemagglutination activity of fungi.

Species No.	Protein/fresh weight of fruit bodies (mg/g)	Hemagglutination activity							
		Titer/g sample (fresh weight)				Titer/mg protein			
		A	B	O	R	A	B	O	R
Eubasidiomycetes									
Agaricales									
1	4.2	710	710	1400	710	170	170	340	170
2	3.7	1600	400	400	6400	430	110	110	1700
4	3.8	17	17	17	1100	4.5	4.5	4.5	290
6	5.2	74	37	18	2200	14	7.1	3.6	450
10	11.1	42	42	42	42	3.8	3.8	3.8	3.8
11	22.7	330	330	330	670	14	14	14	29
12	7.6	17	17	17	70	2.3	2.3	2.3	9.2
13	13.5	nd	nd	nd	850	nd	nd	nd	62
14	10.1	42	42	42	5200	4.2	4.2	4.2	520
15	4.7	110	26	53	6800	22	5.4	11	1500
16	11.7	10	10	10	64	0.9	0.9	0.9	5.5
17	5.6	170	170	350	2800	30	30	60	500
18	8.1	80	80	80	30000	9.9	9.9	9.9	3700
19	1.4	nd	nd	nd	31	nd	nd	nd	22
21	6.5	54	54	110	860	8.3	8.3	17	130
22	2.6	nd	4.1	4.1	8.2	nd	1.6	1.6	3.1
23	3.2	1200	2500	1200	120000	370	780	370	37000
24	8.8	1100	260	260	17000	120	30	30	1900
25	5.5	100	50	50	200	18	9	9	36
26	1.5	125	125	125	63	80	80	80	40
27	7.2	82	82	82	5300	11	11	11	730
28	5.4	290	72	140	1200	54	13	27	210
29	6.2	210	210	210	53000	33	33	33	8400
32	1.0	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
34	3.9	nd	nd	nd	8000	nd	nd	nd	2000
35	19.1	530	260	530	420000	28	14	28	22000
36	17.7	180	180	180	1500	10	10	10	83
37	0.6	nd	nd	nd	110	nd	nd	nd	180
38	1.1	3.0	1.5	1.5	12	2.8	1.4	1.4	11
39	2.4	340	5.3	nd	21	140	2.2	nd	8.8
40	9.1	nd	24	24	1600	nd	2.6	2.6	170
41	23.5	3900	990	990	3900	170	42	42	170
42	4.6	600	150	150	300	130	33	33	66
43	15.5	100	200	100	1600	6.5	13	6.5	100
44	5.3	330	170	1300	11000	63	31	250	2000
45	3.0	40	40	40	2100	13	13	13	670
46	4.9	23	nd	nd	370	4	nd	nd	76
47	13.0	9.1	9.1	18	9.1	0.7	0.7	1.4	0.7
48	5.5	180	360	360	360	33	66	66	66
49	4.2	2700	670	330	10	640	160	80	2.5
51	0.8	4.4	8.8	4.4	8.8	5.5	11	5.5	11
52	2.5	42	21	21	85	17	8.5	8.5	34
53	1.8	40	40	40	2700	22	22	22	1500
54	6.5	140	140	140	19000	22	22	22	2900
55	5.0	nd	10	21	nd	nd	2.1	4.2	nd
56	3.4	nd	nd	nd	50	nd	nd	nd	15
58	2.2	52000	1600	3200	26000	24000	750	1500	12000
59	6.2	nd	nd	nd	16	nd	nd	nd	2.6

Table 2. continued

60	6.6	18	18	9.2	4.6	2.8	2.8	1.4	0.7
62	7.5	nd	nd	nd	160	nd	nd	nd	21
63	2.2	nd	nd	nd	15	nd	nd	nd	6.6
64	6.4	14	7.2	14	57	2.2	1.1	2.2	9.0
65	10.2	70	70	70	7600	6.8	6.8	6.8	750
67	5.4	nd	nd	nd	170	nd	nd	nd	32
68	2.7	4.6	4.6	4.6	4.6	1.7	1.7	1.7	1.7
Aphyllophorales									
69	4.1	50	nd	nd	1600	24	nd	nd	390
70	3.4	150	74	74	19000	43	22	22	5600
71	1.0	62	31	62	125	62	31	62	125
72	8.8	nd	nd	nd	30	nd	nd	nd	3.4
73	9.7	nd	nd	nd	440	nd	nd	nd	45
74	3.2	nd	nd	nd	160	nd	nd	nd	50
75	6.6	25	25	50	100	3.7	3.7	7.4	15
76	2.0	nd	nd	nd	20	nd	nd	nd	10
77	1.0	nd	nd	nd	180	nd	nd	nd	180
78	2.1	2.7	2.7	2.7	2.7	1.3	1.3	1.3	1.3
79	23.7	2400	24000	19000	24000	99	99	800	10000
80	8.3	110	110	110	7000	13	13	13	840
81	17.1	220	450	900	3600	13	26	52	210
82	0.7	13	13	25	13	19	19	39	19
83	7.2	2000	500	500	2000	270	69	69	270
84	21.7	60	30	60	1100	2.8	1.4	2.8	50
85	12.4	h	h	h	5120	h	h	h	410
86	2.8	420	420	840	14000	140	140	280	4700
87	13.7	5600	22000	22000	180000	410	1600	1600	13000
88	2.1	7.8	7.8	7.8	31	3.7	3.7	3.7	15
89	7.6	nd	nd	nd	910	nd	nd	nd	120
90	0.8	1.1	1.1	1.1	4.4	1.3	1.3	1.3	5.2
92	3.1	nd	nd	nd	18000	nd	nd	nd	6000
93	17.3	9400	2300	4600	38000	540	130	260	2100
94	0.5	5.0	5.0	5.0	10	11	11	11	21
Gasteromycetidae									
96	28.3	82	82	82	660	2.9	2.9	2.9	23
97	22.5	22	11	22	90	1.0	0.5	1.0	4.0
98	1.9	3.0	3.0	3.0	6.0	0.6	0.6	0.6	1.2
99	3.3	nd	nd	nd	83	nd	nd	nd	25
100	10.3	nd	nd	nd	18	nd	nd	nd	1.7
101	3.5	nd	nd	330	330000	nd	nd	95	95000
102	2.8	160	160	1300	1300	59	59	480	480
Heterobasidiomycetes									
104	2.9	38	38	38	38	13	13	13	13
105	15.4	nd	nd	nd	400	nd	nd	nd	26
Ascomycotina									
106	22.8	23	23	23	180	1.0	1.0	1.0	8.0
107	0.9	89	44	180	6800	100	52	211	8100
108	1.2	11	11	11	88	8.7	8.7	8.7	70
109	1.6	580	580	2300	4600	350	350	1400	2800
110	11.4	nd	nd	nd	94000	nd	nd	nd	8200

Numbers in this table correspond to those in Table 1. A, B, O and R are the results with trypsinized human A, B, O and rabbit erythrocytes, respectively.

nd.: activity was not detected at 1 mg protein/ml. h: hemolysis was observed.

No. of the species exhibiting no activity and their protein contents (mg/g fresh weight); 3, 3.1; 5, 2.6; 7, 10.6; 8, 6.8; 9, 2.4; 20, 1.8; 30, 8.6; 31, 0.6; 33, 0.3; 50, 6.0; 57, 4.9; 61, 0.6; 66, 7.5; 91, 8.9; 95, 0.8; 103, 3.2.

was higher than reported previously (at most 50%).

In the present study, we used trypsin-treated erythrocytes. Generally, proteinase-treated erythrocytes agglutinate more readily than native erythrocytes. Furthermore, when the crude extracts showed no hemagglutination, the activity was measured in solutions concentrated with $(NH_4)_2SO_4$. These are presumably the reasons why the higher proportion of active species was found.

Blood group specificity Some species exhibited blood group specific hemagglutination. A-type blood specific hemagglutination was found in the extracts of *Panellus serotinus* (Pers.: Fr.) Kühn (39), *Psathyrella piluliformis* (Bull.: Fr.) P. D. Orton (46), *Cantharellus cibarius* Fr. (69) and *Stropharia rugosoannulata* Farlow (58). The extract of *P. serotinus* (Pers.: Fr.) Kühn (39) exhibited weak hemagglutination of B-type erythrocytes. The extract of *S. rugosoannulata* exhibited very strong hemagglutination of A-type erythrocytes and weaker hemagglutination of B- and O-type erythrocytes. Hemagglutination of B- and O-erythrocytes was found in *Gyroporus castaneus* (Bull.: Fr.) Quél. (22) and *Panellus stypticus* (Bull.: Fr.) Karst. (40). O-type specific hemagglutination was observed in *Linderia bicolumnata* (Lloyd) Cunn. (101) and *Phallus impudicus* L.: Pers. (102).

Extracts of 21 species (13, 19, 34, 37, 56, 59, 62, 63, 67, 72–74, 76, 77, 85, 89, 92, 99, 100, 105, 110) were active against only rabbit erythrocytes.

All species that were active against human erythrocytes also agglutinated rabbit erythrocytes. Exceptionally, *Russula violeipes* Quél. (55) agglutinated only human erythrocytes but not rabbit erythrocytes. Similarly, *P. serotinus* (39) and *Rozites caperata* (Pers.: Fr.) Karst. (49) agglutinated human A-type erythrocytes more strongly than rabbit erythrocytes. These extracts should be reinvestigated later.

Distribution of hemagglutinating activity We analyzed 110 species covering 76 genera. Sixty-nine of these species had not previously been analyzed. Twenty-four species were found only in Japan or Japan and Asia (Table 1). Pemberton (1994) showed the results of 404 species covering 142 genera.

Forty-one species (5, 7, 9, 11–15, 22, 23, 27, 33, 36–40, 45, 46, 49, 50, 52, 53, 56, 59–61, 63–65, 69, 78, 84, 86, 89, 92, 100, 102, 103, 108, 110) in this study have been already surveyed, but only 9 species (27, 38, 50, 52, 61, 64, 65, 102, 103) gave almost the same results as those previously reported. Nineteen species (14, 15, 22, 36, 37, 39, 40, 46, 49, 56, 59, 60, 63, 69, 78, 84, 89, 92, 100) that gave negative results in some investigations (Coulet and Merland, 1960a, b; Coulet et al., 1964; Pemberton, 1994; Seeger and Wiedmann, 1972) exhibited hemagglutination in the present study. On the contrary, 3 species (7, 9, 33) that previously showed activity (Pemberton, 1994) were found to be negative. These results suggest that the presence of lectin in some of these species will depend on the sample.

Coulet and Merland (1960a) showed the variation of hemagglutination in some *Amanita* species including *A. rubescens* Pers.: Fr. (5) and *A. vaginata* (Bull.: Fr.) Vitt. (7), and Pemberton (1994) pointed out that this was caused by geographical distribution of each fungus. It is apparent that some of the discrepancies with the previous results were due to variation within each species.

Generally, hemagglutination was not commonly found in *Russula* species, and no active *Suillus* species were reported. In this study, however, only *Russula emetica* (Schaeff.: Fr.) S. F. Gray (50), one of six *Russula* species (50–55), was inactive, and *Suillus luteus* (L.: Fr.) S. F. Gray (61), one of three *Suillus* species (59–61), did not exhibit hemagglutination.

It has been reported that hemagglutination activity was rarely detected in the extracts of Gasteromycetes, Heterobasidiomycetes, and Ascomycetes other than the Pezizales. Table 2 shows, however, that most species of these groups show hemagglutinating activity. Of eight gastoromycete species, only *Astraeus hygrometricus* (Pers.) Morgan (95) was inactive. Of three heterobasidiomycete species, only *Tremella foliacea* Pers.: Fr. (103) was inactive. We previously purified and characterized a lectin from cultured fruit bodies of *Auricularia polytricha* (Mont.) Sacc. (Yagi and Tadera, 1988). All five Ascomycete species were active in this study. These results suggest that lectins are present in most genera and families of fungi.

Comments on the fungal lectin

We surveyed fungal lectins in fruit bodies. However, lectins were also detected in mycelia (Yatohgo et al., 1988; Kawagishi et al., 1997), and some mycelial lectins were purified to homogeneity. The relationship of lectins in fruit bodies and mycelia is not yet well understood.

In plants, several lectin families (protein groups with homologous amino acid sequences developed from a common ancestor) are known (Rini, 1995; Wright, 1997). Similarly, several lectin families can be assumed for fungi. At present, three lectin families are known for fruit bodies of fungi. One family is the immunomodulatory proteins, which were found in *G. lucidum* (Tanaka et al., 1989), *Flammulina velutipes* (Curt. Fr.) Sing. (Hashimoto, 1990), and *Volvariella volvacea* (Bull. ex Fr.) Singer (She et al., 1998). Their molecular masses were 13–16 kDa. The second is represented by the 34-kDa *A. aurantia* lectin (Fukumori et al., 1990), of which the primary structure has been determined. Thirdly, Cooper et al. (1997) reported two galectins from *Coprinus cinereus* (Schaeff.: Fr.) S. F. Gray, a type universally found in the animal kingdom. No sequence homology was observed among the structures of these three families of lectins.

In future, in order to clarify the number and distribution of lectin families in fruit bodies, it will be necessary to determine the structures of many more fungal lectins.

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