Microbes Environ. Vol. 19, No. 3, 227–235, 2004
http://wwwsoc.nii.ac.jp/jsme2/

Microbes and Environments

Lectin Variation in Members of *Rhizoctonia* Species

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(Received November 11, 2003—Accepted March 27, 2004)

Isolates from two multinucleate *Rhizoctonia* species, namely *Rhizoctonia solani* and *Rhizoctonia circinata*, and binucleate *Rhizoctonia* spp., were evaluated for the presence of lectins, using human erythrocytes. Isolates from *R. solani* had similar lectins across the anastomosis groups. Agglutination profiles, however, revealed individual preferences for human blood types but with a general preference for type A over B and O. *R. solani* lectins had a general affinity for *N*-acetyl-D-galactosamine and mucin. Also, some isolates from the binucleate *Rhizoctonia* AG-DII, had lectins that showed strong affinity for glycoproteins, fetuin and mucin. There was no lectin activity in members of *R. circinata*. The lectins in members of the genus *Rhizoctonia* varied in content and character across the species sampled but had similar affinity for mucin. Furthermore, 62% of the self-anastomosing and 35% of the non-self anastomosing isolates tested showed lectin activity. The presence of lectins in both self-anastomosing and non-self anastomosing isolates suggests that lectins may not be directly involved in the recognition process in hyphal anastomosis.

Key words: *Rhizoctonia* spp., lectin variation, anastomosis

Rhizoctonia fungi are known to be pathogenic to a wide range of host plants. The genus *Rhizoctonia* is divided into uninucleate, binucleate and multinucleate types with *R. solani* and *R. circinata* being multinucleate types. Isolates of *Rhizoctonia* species have been placed further into anastomosis groups (AG) based on their hyphal anastomosis behavior[21]. Members of *R. solani* have been assigned to 13 AGs running from AG 1 through 13[15,16,17,19,21]. On the other hand, isolates of *R. circinata* have been assigned to four varieties based on cultural morphology[22]. Previously, isolates of binucleate *Rhizoctonia* (BNR) except AG-Q had been assigned to 20 AGs from AG-A through AG-U[18,20,21]. However, based on fatty acid composition, and RFLP and RAPD analyses, AG-Q had been reassigned to a subgroup of AG-D[20,23]. Also, the presence of clamp connections in isolates of AG-J and AG-M (Ogoshi; personal communication) excludes these two groups from the genus *Rhizoctonia*. This further reduces the AG number to 18. So far, isolates of uninucleate *Rhizoctonia* have been reported in conifer seedlings in Finland and Norway[7].

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Recently, the presence of lectins on the hyphal surfaces and tissues of *Rhizoctonia* fungi has been reported[4,11,12,13,24]. These findings, however, were based on limited groups of *Rhizoctonia* spp. Lectins are sugar-binding proteins that agglutinate cells and precipitate glyco-conjugates[6]. Biologically they are more active as aggregates, generally dimers or tetramers, than monomers and are involved in interactions between the cell surface and extracellular environment[3]. Lectins are highly specific and can discriminate between different types of cells that have only minor variations. Their ability to discriminate between human red blood cells (A, B and O) that have different terminal non-reducing sugars in their major glycoprotein has been used in their partial characterization[25]. Studies have shown that the ability of lectins to bind carbohydrates is directly involved in fungal-

Table 1. Isolates used in this study

Species/Sub-groups	Isolate	Host[source]
R. solani		
AG 1-IA	RRC-97-1	Rice
	G7	Rice
	GN-02-1	Rice
	C54	Rice
	Mong 390	Rice
AG 1-IB	TR 22	Kentucky grass
	R147	White clover
	LF-08-1	European pear
AG 1-IC	F1	Sugar beet
	IR4	Pine
	BW3	Buckwheat
	189	Cauliflower
	P1	Sugar beet
AG 1-ID	RCP-1	Coffee[a]
	RCP-3	Coffee[a]
	RCP-7	Coffee[a]
AG 2-1	B-1	Nd[b]
	SH-10	Soil
	SH-20	Soil
AG 2-2IIIB	PGR-01-1	Bent grass
	AS-5-S-IS	Nd[b]
	Gu-1	Guzmania
	AK 96-7	Nd[b]
	K2-8	Bent grass
AG 2-2IV	SA-1	Sugar beet
	P 1211	SA-1 progeny
	H16	Sugar beet
	R94	Sugar beet
	Rh 509	Sugar beet
	92155	Sugar beet
	S-2	Sugar beet
AG 2-t	2TR102	Tulip[c]
	2TR103	Tulip[c]
	2TR 116	Tulip[c]
	2TR 117	Ixia[c]
	2TR122	Tulip[c]
	2 TR134	Tulip[c]
	2TR 137	Tulip[c]
AG 2-2LP	M16-h	Zoysia grass
	AJ1-10-1	Zoysia grass
	C4-h	Zoysia grass
	M10-d2	Zoysia grass
	M1-m	Zoysia grass
	M 23-h	Zoysia grass
AG 2-3	R-4	Soybean
	H5-316	Soybean
	H5-354	Soybean
	R1	Soybean
	R8	Soybean
	H4-30	Soybean
AG 2-BI	SF-BV-1	Soil
	SHC-81	Soil
AG 3	P-5	Potato
	1Sal3	Potato
	1Sal 4	Potato
	SR-5	Nd[b]
	SP-5	Potato[d]
	SP-15	Potato[d]
	R468	Potato
AG 4	521-21	Soil
	AH-1	Peanuts
	Rh165	Sugar beet
	MWR5-2	Sugar beet
AG 5	Rh184/S-1	Sugar beet
	KF 722-20	Nd[b]
	B-81	Soil
	SH-1	Soil
	R189	Nd[b]
	AG95-12	Nd[b]
AG 6	FK1-1-41	Nd[b]
	AO1-7	Soil
	NKN 2-1	Soil
	YK 2-3	Nd[b]
	YK 3-3	Nd[b]
	OT 2-1	Soil
AG 7	1529	Soil
AG 8	A21	Wheat[e]
	A125	Wheat[e]
	SA124	Barley[e]
	C1	Barley[f]
	SA1512	Oat[e]
BNR[g] spp.		
AG-A	AH-1	Peanuts
	YS-6-4-2A	Nd[b]
	C538	Potato
AG-Ba	C460	Rice
	C484	Rice
	C-314	Rice
	C-315	Rice
AG-Bb	C-455	Rice
	C-350	Rice
AG-C	Ka-1-1	Soil
AG-DI	TG-SDS-1	Zoysia grass
	WP 4	Zoysia grass
	W-12	Zoysia grass
	BrG-Wp-2	Zoysia grass
	WG-IB	Zoysia grass

AG-DII	OK-EF-1	Rice
	WK-EF-1	Rice
	KT-1-1	Rice
	YG-EF-1	Rice
	YC-EF-1	Rice
AG-E	OC-1	Woodsorrel
	Rh-155	Soybean
	LU-1	Flax
AG-F	SIR-1	Sweet potato
	A-1-16	Nd[b]
AG-G	Su-1	Chickweed
	AH-9	Peanuts
	C393	Nd[b]
	N14	Nd[b]
AG-I	AV-2	Sage brush
AG-K	SH-10	Soil
AG-L	FKO-2-26	Soil
AG-O	FKO-6-2	Soil
AG-P	C-578	Tea
AG-R	BN-37	Cucumber
AG-S	S5	Soil
AG-T	MWR-26	Rose flower
AG-U	HOK-07-1	Miniature rose
	99-RM-1	Miniature rose
	5Oit-800	Miniature rose
	4Oit-800	Miniature rose
	1FUK600	Miniature rose
	2NIG2-600	Miniature rose
R. circinata		
var. *circirnata*	HHC-13-6	Bentgrass
	HTB-A-I	Bentgrass
	MAR-BG	Bentgrass
	KT-5S-1	Bentgrass
	AMI-BG	Bentgrass
	KOUCH-BG	Bentgrass
var. *agrostis*	HMA-BG	Bentgrass
	HIG-BG-A	Bentgrass
	NANZ-BG-A	Bentgrass
	INAS-BG	Bentgrass
	NUK-BG	Bentgrass
	HIG-BG-B	Bentgrass
var. *oryzae*	GH-700	Zoysia grass
	00-103B	Zoysia grass
	Mm 9	Zoysia grass
	Mm 4-3	Zoysia grass
var. *zeae*	C504	Soil
	M-003	Soil
	As7s11A	Nd[b]
	11sh	Nd[b]

[a] Philippines, [b] No data, [c] Netherlands, [d] Spain, [e] Australia, [f] USA, [g] Binucleate *Rhizoctonia* spp.

mycoparasite interactions[1,2,4,9]. The detection of lectins on hyphal surfaces of some *Rhizoctonia* spp. also raises the question of whether their presence is related to the recognition process in hyphal anastomosis.

Hyphal anastomosis is spontaneous and seems to be genetically controlled[5]. Perfect fusions are observed only among hyphae originating from a common isolate or plug, while fusion between related isolates results in plasmolysis of fused cells. On the other hand, hyphae from unrelated isolates can only achieve contact fusion, which is not considered anastomosis[21]. Studies have shown that some isolates self anastomose while others do not[8,14,26,27]. In this study, the presence of lectins in all the known members of the genus *Rhizoctonia* was investigated and the type of lectins in each species was partially characterized. Also, the distribution of lectins in self-anastomosing isolates (SAIs) and non-self anastomosing isolates (NSAIs) was analyzed and its relationship with the anastomosis phenomenon is discussed.

Materials and Methods

Isolates used in this study

A total of 144 isolates from *Rhizoctonia solani, R. circinata* and binucleate *Rhizoctonia* spp. was sampled. Of the sampled isolates, 81 were from *R. solani*, 20 from *R. circinata*, and 43 were from binucleate *Rhizoctonia* spp. (Table 1).

Extraction of crude lectins

Mycelia were grown for 16 days at 25°C on potato dextrose broth in static cultures. Agar discs (5 mm, diameter) covered with mycelia were cut from the margins of 5-day old cultures grown on potato dextrose agar (PDA) at 25°C and used as inocula. Mycelia were harvested then homogenized using a mortar and pestle in phosphate-buffered saline (PBS-pH 7.4; 8 g Nacl, 2.9 g Na_2HPO_4. $12H_2O$, 0.2 g KCl, 0.2 g KH_2PO_4 in 1liter of Milli-Q water). Crude lectins (CL) were then extracted overnight in PBS at 4°C, and centrifuged at 8000 rpm, 4°C for 15 min (Tomy-RS 206, Tomy Digital Biological Co., Tokyo, Japan). The supernatants were collected as CL that were then used for hemagglutination and inhibition assays or stored at −80°C prior to use.

Lectin detection

Human blood was collected in 3% sodium citrate. The erythrocytes were washed three times in PBS and suspended at a 3% concentration in the buffer. A 10% suspension of erythrocytes in PBS (10 ml) was treated with 7 mg of pro-

nase (CALBIOCHEM, La Jolla, Canada) for 30 min at 47°C. The erythrocytes were then washed three times in PBS and suspended at a concentration of 3% in the buffer. Agglutination of 3% erythrocytes and inhibition of the agglutination by sugars and glycoproteins were conducted in 96-well microtiter U-plates (Nalge Nunc International, New York, NY, USA). The titer was defined as a reciprocal of the end-point dilution causing hemagglutination[10].

Lectin activity was detected using both treated and untreated blood. The intensity of activity was scored based on hemagglutination patterns that were monitored visually. Agglutination at titers 2^1–2^4, 2^5–2^8, and 2^9–2^{11} of CL was considered to reflect low (+), moderate (++) and high (+++) lectin activity, respectively (Fig 1). Lack of agglutination (–) indicated that the samples had no lectin activity. For comparison, untreated erythrocyte suspensions were also used.

Carbohydrate inhibition assays

Two-fold serial dilutions of 10 μl of test monosaccharides (400 mM) or glycoprotein (1 mg ml^{-1}) were made in 10 μl of PBS across the U-plates. The dilutions were then incubated with 10 μl of titer 2^4 CL at room temperature for 1 hour. The setups were then overlaid with 20 μl of 3% human erythrocytes, incubated for 30 minutes and then observed. Inhibition was expressed as the minimum concentration of each monosaccharide or glycoprotein that was required to inhibit hemagglutination at a titer of 2^4 CL. Lack of agglutination was therefore considered inhibition at that monosaccharide or glycoprotein concentration. Inhibition at titers 2^1–2^4, 2^5–2^8 and 2^9–2^{11} was considered to reflect respectively, low (+), moderate (++), and high (+++) affinity by the test CL for the carbohydrate tested as indicated in Fig. 2. The tests were repeated at least three times.

Self-anastomosis tests

Plugs (5 mm diameter) from the margins of 3 to 5 day old PDA cultures were paired opposing each other (2 cm) on glass slides coated with a thin layer of 2% water agar (WA). The slides were then incubated under high humidity at room temperature for 2–3 days until hyphal tips made contact. Observations were made under the microscope (×200) after staining with 0.1% cotton blue in lactophenol. The ability of each isolate to self-anastomose or not was determined by observing the hyphal contact points that achieved fusion. Fusion frequencies (FF) were then calculated using the formula, (FF=A (100)/B), where A is the sum of fused contact points and B the sum of contact points in 15 microscopic

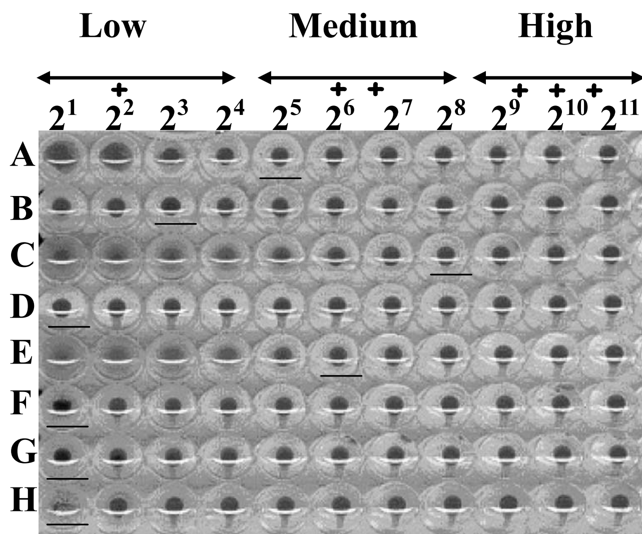

Fig. 1. Lectin detection range using pronase-treated erythrocytes. A-H corresponds to crude lectins (CL) from different isolates tested and line 2^1–2^{11} to the CL dilution levels (titers). The underbars represent lectin activity levels for each test CL. Agglutination levels at titer 2^1–2^4, 2^5–2^8 and 2^9–2^{11} were scored as low (+), moderate (++) and as high (+++) lectin intensity, respectively.

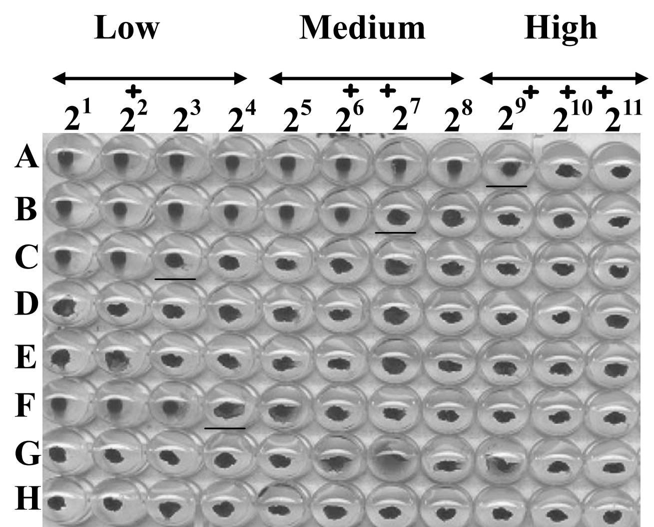

Fig. 2. Affinity range for carbohydrates in the inhibition assays. A 96-well micro-titer U-plate showing the results of carbohydrate inhibition assays using pronase-treated human erythrocytes. A-H corresponds to carbohydrates (GalNAc, galactose, D-fucose, glucose, manose, mucin, asialo-fetiun and fetuin, respectively) against AG 1-IC crude lectins of titer 2^4. Line 2^1–2^{11} indicate the carbohydrate dilution levels (titers). Hemagglutination between CL and human erythrocytes was inhibited at titers 2^9, 2^7, 2^3 and 2^4 (indicated by the underbars) by GalNAc, galactose, D-fucose and mucin, respectively. Inhibition at titers 2^1–2^4, 2^5–2^8 and 2^9–2^{11} was scored as low (+), moderate (++) and high (+++) affinity, respectively.

Table 2. Composition and character of isolates used in this study

Species	Number of samples	Frequency of occurrence (%)		Lectin occurrence in (%)		
		SAIs[a]	NSAIs[b]	All samples	SAIs	NSAIs
R. solani	81	88	12	83	86	60
R. circinata	20	100	0	0	0	—
BNR spp.[c]	43	82	18	7	9	0
Rhizoctonia species.	144	88	12	49	62	35

[a] Self-anastomosing isolates.
[b] Non-self-anastomosing isolates.
[c] Binucleate *Rhizoctonia* spp.

fields. Fusion frequencies less than 30% were considered low (+), those between 30 and 50%, moderate (++), and those more than 50%, high (+++)[21)].

Results and Discussion

Self-anastomosis phenomenon

The ability to self-anastomose in isolates of *R. solani* varied from an inability to anastomose to high fusion frequencies across the AGs (data not shown). Out of the 81 isolates sampled from *R. solani,* 88% were SAIs (Table 2). The occurrence of NSAIs was 12% in *R. solani*. NSAIs were predominantly (50%) from AG 2. Similarly, AG 5, AG 6 and AG 8 had some NSAIs. On the other hand, all the 20 isolates sampled from *R. circinata* were SAIs (Table 2). Fusion frequencies in binucleate *Rhizoctonia* spp. were low for most isolates. However, isolates of AG-DI had high FF values compared to other AGs in this species (data not shown). The occurrence of NSAIs in this species was 18% (Table 2).

Lectin activity

Lectin activity in members of *R. solani* ranged from none in isolates of AG 2-2IIIB to high intensity in those of AG 1-IA, AG 2-2IV and AG 2-BI (Table 3). These lectins, however, were closely related in their preference for human blood type A over type B and O. *R. solani* agglutinins (RSA) had affinity for GalNAc (0.1 mM–100 mM), galactose (0.8 mM–100 mM) and D-fucose (13 mM–100 mM) in that order of preference across the AGs. Also, lectins from all sampled AGs had affinity for a glycoprotein, mucin, at concentrations ranging from 0.8 $\mu g\ ml^{-1}$ to 250 $\mu g\ ml^{-1}$ across the AGs. Affinity levels for carbohydrates generally varied across the AGs in *R. solani* (Tables 3 and 4). Interestingly, the 20 isolates sampled from *R. circinata* had no lectin activity at all (Table 2).

Only 7% of the isolates from binucleate *Rhizoctonia* spp. had lectin activity. These isolates belonged to AG-DII. The intensity, however, was low in this genus (Table 3). The occurrence of lectins in SAIs of *R. solani* was 86% while that in NSAIs was 60% (Table 2). However, in binucleate *Rhizoctonia* spp., lectin was detected in 9% of the SAIs. None of the NSAIs in this genus had lectins (Table 2). Out of all *Rhizoctonia* isolates sampled, 62% of the anastomosing and 35% of the non-anastomosing isolates had lectins detected at varying concentrations (Table 2).

Lectin activity in *Rhizoctonia* fungi varied sharply across the species with *R. solani* having intense activity and a few binucleate *Rhizoctonia* spp. having weak activity. Our results concur with those of Kellens *et al*. (1991) who reported no lectins in members of *R. circinata*[13)]. Agglutination profiles in RSA revealed a close relationship among anastomosis groups of *R. solani*. There was a general preference for blood type A over B and O. Certain isolates within and among AGs, however, exhibited individual preferences with some isolates from AG 1-IB and AG 7 having preference for blood type A only, while AG 2–3 preferred blood type O over A and B (Table 3). Agglutination profiles, however, did not conclusively divide the *R. solani* isolates into their distinct AGs but rather expressed some relatedness among them. This relatedness is also expressed by the common affinity for GalNAc and mucin.

The distinct absence of lectins in members of AG 2-2IIIB and some members of AG 6, however, marked a major variation in lectin activity in this species. AG-DII isolates from binucleate *Rhizoctonia* spp. had lectins with agglutination profiles (A over B and O) similar to RSA in blood type preference (Table 3). These lectins, however, had affinity for only glycoproteins, fetuin and mucin. From the agglutination profiles, AG-DII lectins show some relationship to RSA but are distinctly different in their specificity for carbohydrates. Generally *Rhizoctonia* lectins had preference for human blood type A over B and O and had affinity for mucin. None of the *Rhizoctonia* lectins tested had affinity for glucosamine, *N*-acety-D-glucosamine, nor

Table 3. Lectin variation in *Rhizoctonia* species

AG	No. of isolates sampled	Lectin activity		Blood type preference	Carbohydrate affinity	
		Frequency (%)	Intensity		Monosaccharides	Glycoprotein
R. solani						
AG I-IA	5	100	+++	A>O>B	GalNAc>Gal	Mucin
AG 1-IB	3	100	++	A	GalNAc,Gal>D-fuc	Mucin>Fet[a]
AG 1-IC	5	100	+	A>B>O	GalNAc>Gal	Mucin
AG 1-ID	3	100	++	A>O>B	GalNAc	Mucin
AG 2-1	3	100	++	A, B>O	—	Mucin
AG 2-2IIIB	5	0	−	—	—	—
AG 2-2IV	7	100	+++	A>O>B	GalNAc	Mucin
AG 2-2LP	6	100	++	A, O>B	—	Mucin
AG 2-t	7	100	++	A, B, O	—	Mucin
AG 2-3	6	100	++	O>A>B	GalNAc	Mucin
AG 2-BI	2	100	+++	A>B>O	GalNAc,Gal	Mucin
AG 3	7	86	++	A>O>B	—	Mucin
AG 4	4	75	++	A>B>O	GalNAc>Gal,D-Fuc	Mucin
AG 5	6	67	+	A>B>O	—	Mucin
AG 6	6	17	±	A>O>B	—	Mucin
AG 7	1	100	+	A	GalNAc>Gal	Mucin
AG 8	5	100	++	A>B>O	GalNAc	Mucin>Fet
R. circinata[b]						
var. circinata	6	0	—	—	—	—
var. agrostis	6	0	—	—	—	—
var. oryzae	4	0	—	—	—	—
var. zeae	4	0	—	—	—	—
Binucleate *Rhizoctonia* spp						
AG-A	3	0	—	—	—	—
AG-Ba	4	0	T[c]	A	—	—
AG-Bb	2	0	—	—	—	—
AG-C	1	0	—	—	—	—
AG-DI	5	0	—	—	—	—
AG-DII	5	60	+	A, B>O	—	Fet>Mucin
AG-E	3	0	—	—	—	—
AG-F	2	0	—	—	—	—
AG-G	4	0	—	—	—	—
AG-I	1	0	—	—	—	—
AG-K	1	0	—	—	—	—
AG-L	1	0	—	—	—	—
AG-O	1	0	—	—	—	—
AG-P	1	0	T	A	—	—
AG-R	1	0	—	—	—	—
AG-S	1	0	—	—	—	—
AG-T	1	0	—	—	—	—
AG-U	6	0	—	—	—	—

[a] Fetuin.
[b] All four varieties of *R. circinata* tested were SAI without lectins.
[c] Traces.

Table 4. Carbohydrates specificity of RSA[a] and BNR[b] lectins

AG	Monosaccharides (mM)							Glycoprotein (μg ml^{-1})		
	GalNAc	GlcNAc	Gal	L-Fuc	D-Fuc	Man	Glc	A/fetuin[c]	Fetuin	Mucin
AG I-IA	1.6	—	25.0	—	—	—	—	—	—	7.5
AG 1-IB	0.4	—	0.8	—	13.0	—	—	1.5	0.2	1.5
AG 1-IC	0.4	—	1.6	—	25.0	—	—	—	—	31.0
AG 1-ID	25.0	—	100.0	—	—	—	—	—	—	16.0
AG 2-1	0.8	—	6.3	—	50.0	—	—	—	—	31.0
AG 2-2IIIB	*[d]	*	*	*	*	*	*	*	*	*
AG 2-2IV	>100.0	—	—	—	—	—	—	—	—	0.8
AG 2-2LP	>100.0	—	—	—	—	—	—	—	—	1.5
AG 2-t	0.4	—	0.8	—	13.0	—	100.0	—	—	31.0
AG 2-3	>100	—	—	—	—	—	—	—	—	16.0
AG 2-BI	1.6	—	13.0	—	100.0	—	—	—	—	250.0
AG 3	>100	—	—	—	—	—	—	—	—	3.6
AG 4	0.4	—	6.3	—	25.0	—	—	—	—	31.0
AG 5	>100.0	—	—	—	—	—	—	—	—	16.0
AG 6	>100.0	—	—	—	—	—	—	125.0	—	1.5
AG 7	0.1	—	1.6	—	—	—	—	—	—	1.5
AG 8	50.0	—	—	—	—	—	—	125.0	250.0	1.5
AG-DII[b]	—	—	—	—	—	—	—	—	0.8	7.5

[a] *Rhizoctonia solani* agglutinins from AG 1 to AG 8.
[b] Binucleate *Rhizoctonia* (AG-DII) lectins.
[c] Asialo fetuin.
[d] No data due to lack of lectins in members of the AG. The values in the table represent the minimum concentrations of monosaccharides (mM) and glycoprotein (μg ml^{-1}) that inhibited hemagglutination to RSA and BNR lectins. Low values indicate high affinity of the test CL for the respective test carbohydrate. The titer of RSA and BNR lectins used in this test was 2^4.

L-fucose (Table 4).

The involvement of lectins in the fungus-mycoparasite and cell-cell recognition process has recently been elucidated[2,4,9]. Elad *et al*. (1983) suggested that lectins on the surfaces of *R. solani* act as an elicitor, while the galactose molecules on *Trichoderma harzianum* hyphae respond (receptor) to stimuli in a fungus-mycoparasite interaction. Similarly, Inbar and Chet (1992) demonstrated using nylon fiber biomimics, that lectins are directly involved in the recognition process of the mycoparasitic fungus, *T. harzianum* to *Sclerotium rolfsii*[9]. The presence of lectins on hyphal surfaces of *Rhizoctonia* fungi suggests that they may also be involved in the recognition process in hyphal anastomosis. Ideally, SAIs should have both elicitor and receptor sites along the hyphae as hypothesized by Kuninaga (1980)[14] and Yokoyama *et al*. (1985)[26,27]. A lack of either one of these will, therefore, result in the inability of that hyphae to self-anastomose.

Most patterns of anastomosis are mediated from the tips of the hyphae although there are cases of side-by-side contacts that also result in fusion. In our experiments, both SAIs and NSAIs clearly showed the presence of lectins on hyphal surfaces when challenged with pronase- treated human erythrocytes (Figs. 3a and 3b). Binding was distinctly noted on the older cells of the hyphae rather than the growing tips. In contrast, isolates from *R. circinata* and some from binucleate *Rhizoctonia* spp. and *R. solani* that anastomosed at different frequencies did not have any lectins at all. Furthermore, tests using various monosaccharides as lectin inhibitors in membrane bioassays did not reveal any significant change in the fusion frequencies of SAIs nor NSAIs (data not shown).

Media containing L-asparigine have been reported to strongly affect the lectin content in *R. solani* cultures[12]. The introduction of L-asparagine monohydrate (4 g L^{-1}) in 2% WA bioassay setups had a significant increase on the FF of SAIs but had no effect on that of NSAIs. This change was possibly nutritional because extensive hyphal branching was noted for both NSAIs and SAIs. Consequently, more hyphal contact points and subsequently more fusions were

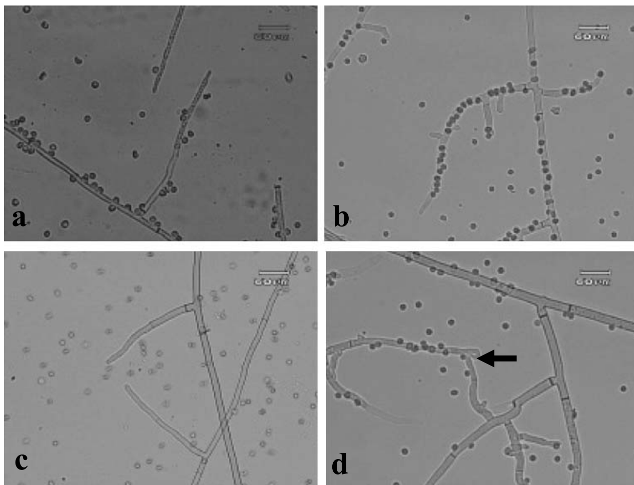

Fig. 3. Lectin deposition sites on hyphae of a SAI and NSAI from *Rhizoctonia solani* AG 2-2IV. Continued from LIST OF FIGURES. a: Hyphae of isolate H16 (NSAI) challenged with pronase-treated human erythrocytes. Erythrocyte attachment points indicate lectin deposition sites. b: Hyphae of isolate SA-1 (SAI) showing more lectin sites on older hyphal cells compared to the growing tips. A similar phenomenon is observed in Fig. 3a. c, d: show SA-1 hyphae at the recognition stage (c) and after hyphae have fused (d). The deposition of lectin on the hyphal surfaces is not concentrated at the fusion sites during anastomosis.

realized for SAIs but no fusion occurred in NSAIs (data not shown).

The *Rhizoctonia* lectins isolated to date have been characterized as dimers or tetramers with rare cases of monomers, suggesting that they are more active as aggregates and do play a major biological role in the species[1,3,11,12,13,24]. Although lectin on the hyphal surfaces of *Rhizoctonia* fungi have previously been demonstrated to be a fungal-fungal recognition component, preliminarily, our data did not correlate lectin with the anastomosis recognition process. However, the close similarity in carbohydrate specificity and preferential affinity for human blood type A in *Rhizoctonia* lectins demonstrates a close relationship among lectins from this species. Individual specific characters, however, are occasionally exhibited. To the best of our knowledge this is the first report characterizing lectins from binucleate *Rhizoctonia* spp.

Acknowledgements

The authors are grateful to M. Julian, S. Neate, D.E. Carling, J.H.M. Schneider and C. Pascual for providing isolates of *R. solani.* Members of the Plant Pathology Laboratory, Gifu University, are acknowledged for their invaluable support through blood donations, while O.-A. Siaw is acknowledged for his comments and critical reading of the manuscript. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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