Studies on the Anastomosis Groups of *Rhizoctonia solani* Kühn

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From many studies of imperfect and perfect stages, *Rhizoctonia solani* (*Thanatephorus cucumeris*) has been recognized as being characterized by; 1) Multinucleate cells in young vegetative hyphae, 2) Prominent septal pore apparatus (dolipore septum), 3) Branching near the distal septum of cells in young vegetative hyphae, 4) Constriction of the branch and formation of a septum in the branch near the point of origin, 5) Some shade of brown, 6) The perfect stage is *T. cucumeris*, 7) No clamp connections, 8) No conidia, 9) No sclerotia differenciated into a rind and medulla, 10) No rhizomorphs, 11) No red, green, blue, blight yellow, orange, or other pigment except brown.

Many fungi that have previously been assigned to *R. solani* would now be excluded with the above items. Therefore, earlier reports on *R. solani* are subject to reconsideration.

It is widely recognized that *R. solani* consists from many races, forms or groups of various isolates differing in pathogenicity, morphology, and ecology. There are a number of reports on grouping of *R. solani* and those groupings are based on differences of pathogenicity, morphology in culture and/or physiology[1),2),10),11)] and on anastomosis among isolates[5),6),7)].

The present report describes the grouping of isolates of *R. solani* isolated from various plants and soils in Japan with anastomosis. The author's studies of the grouping with anastomosis were already reported in detail[3),4)].

Methods

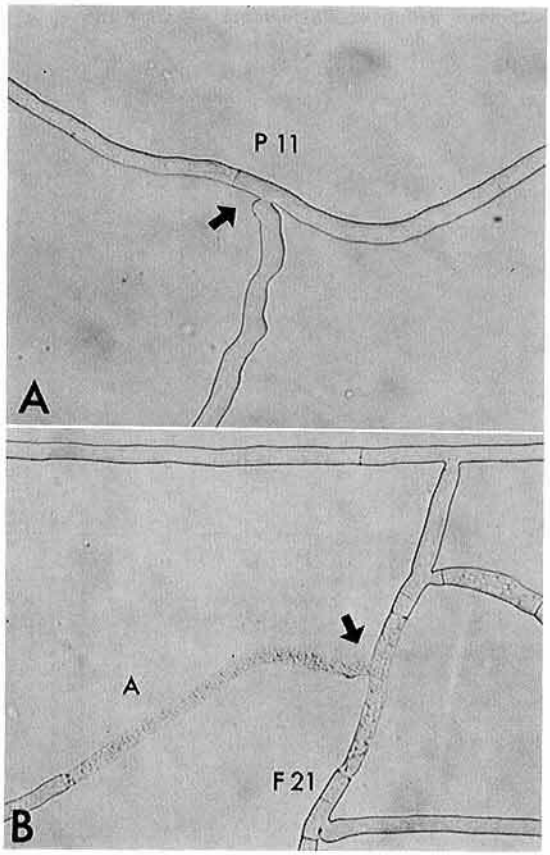
In this study 255 isolates used were isolated from Hokkaido Prefecture in the north to Kagoshima Prefecture in the south.

Anastomosis was tested by opposing isolates on 2% water agar or on cellophane resting on water agar in Petri dishes. Mycelial transfers from the margins of actively spreading young cultures on PDA were incubated at 20°C or room temperature until advancing hyphae made contact and slightly overlapped. This portion was observed under a microscope.

In the course of hyphal contact before the fusion, there were three cases, a) hyphae of two isolates were attracted each other, b) hypha of one isolate was attracted by hypha of another isolate, and c) such attracting phenomena were not observed and fusion was left to chance. Anastomosis within one and the same isolate was perfect fusion (Fig. 1 A). Anastomosis between isolates from the same group was imperfect or contact fusion (Fig. 1 B). It was observed that the imperfect fusion resulted in the death of fused and adjacent cells. Perfect and imperfect fusion mean that these two isolates belong to the same anastomosis group.

Anastomosis groups in Japan

All of 255 isolates except 13 isolates were



A Perfect fusion between two hyphae within one isolate (P11).

B Imperfect fusion between two AG-2 Type-2 isolates (A and F21), showing death of hyphal cells at point of fusion.

Fig. 1. Anastomosis between isolates of *Rhizoctonia solani*

classified into five anastomosis groups (AG-1, AG-2, AG-3, AG-4, and AG-5), and AG-2 was divided into two types by the frequency of anastomosis (AG-2 Type-1 and AG-2 Type-2; Table 1). Table 2 shows highly frequent anastomosis between two isolates from same type in AG-2, but very low frequency between two isolates from Type-1 and Type-2. Thirteen isolates were not assigned any one of groups.

On five anastomosis groups in *R. solani*, hyphal widths, effect of temperatures on mycelial growth, numbers of nucleus in hyphal cell, and colonial appearances were studied comparatively (Table 3).

AG-1 (Plate 1, A) Most of 60 isolates fallen into AG-1 were those from rice plant, sugar beets, and soils. They included sheath blight fungus (*Corticium sasakii*) and web-blight fungus (*C. microsclerotia*). They grew rapidly at 25–28°C, but slightly at 35°C. Among six groups they grew most rapidly (about 30 mm/24 hr) at their optimum temperatures. The tissue of sclerotium was compact.

AG-2 Type-1 (Plate 1, B) Most isolates of AG-2 Type-1 were from soils, flax, and *Cruciferae*. Most of 19 isolates of this group grew rapidly at 23–25°C, but not at 33°C Growth rates were low. On the mycelial colonies of the isolates small reddish-brown sclerotia were formed in concentric zones. Some isolates did not form sclerotia and had abundant aerial mycelia.

AG-2 Type-2 (Plate 1, C) Most of 70 isolates of AG-2 Type-2 were from sugar beets and soils to which sugar beets had been cultivated. Optimum temp. 25–28°C. Their mycelial colonies were dark brown in color and the tissue of sclerotium was loose.

AG-3 (Plate 1, D) All 9 isolates of this group were from *Solanaceae*, mostly potatoes. Most isolates of AG-3 grew well at lower temperature like AG-2 Type-1. Their hyphal widths were widest in six groups. Mycelial colony was resemble AG-2 Type-2.

AG-4 (Plate 1, E) The majority of 42 isolates of AG-4 were from *Legminosae*, sugar beets, and soils. Isolates of AG-4 were able to grow at 35°C and this group was most thermophylic among six groups. Their hyphal widths were narrowest in contrast with AG-3. This group is the "praticola type" (*C. praticola*) and most of their cultural colonies were mealy. GM-4, a isolate of AG-4, has 3.1 nuclei per hyphal cell, contrasting 5–8 nuclei in isolates of other anastomosis groups.

AG-5 (Plate 1, F) The majority of 41 isolates of this group were from soils. Optimum temp. 23–28°C. Growth rates were comparatively low. Hyphal widths were

Table 1. Origins of isolates belonging to each anastomosis group of *Rhizoctonia solani* Kühn

Origin	Anastomosis group						Unassigned isolates	Total
	AG-1	AG-2 Type-1	AG-2 Type-2	AG-3	AG-4	AG-5		
Chanopodiaceae	14		44		9	5	5	77
Leguminosae	5				14	4	2	25
Cruciferae	8	3	6		4	2	1	24
Solanaceae	5			9	2	5		21
Graminae	14	1	3					18
Linaceae		5	1		4	1		11
Unbelliferae			3				1	4
Compositae	1				1	1		3
Rosaceae		2						2
Liliaceae		1				1		2
Other plants	2		3		2			7
Soils	11	7	11		6	22	4	61
Total	60	19	70	9	42	41	13	255

Table 2. Anastomosis between AG-2 Type-1 and AG-2 Type-2

		AG-2 Type-2			AG-2 Type-1	
		Bi-52	R1-2-6	RS-2	R-21	SSa-1
AG-2 Type-1	FC-1	−	−	−	++	++
	SSa-1	+	−	−	++	
	R-21	−	+	−		
AG-2 Type-2	RS-2	++	++			
	R1-2-6	++				

−: Anastomosis was not observed
+: Anastomosis was observed but only 1-2 points in all microscopic field of vision
++: Anastomosis was observed very frequently

Table 3. Some characters of anastomosis groups of *Rhizoctonia solani* Kühn

	Hyphal widths (μm) of isolate tested				Optimum temp. (C) for growth on PDA	Linear growth rate at optimum temp. (mm/24 hr)	Number of nuclei in hyphal cell		
	No. of isolate tested	Min.	Max.	Mean			Range	Mean	Isolate
AG-1	12	7.7	9.9	9.0 a*	25—28	(23-) 30—40	3— 8	5.8	R I-86
AG-2 Type-1	15	7.9	10.1	9.2 a	(20-) 23—25	12—16	3— 9	5.3	H V-1
AG-2 Type-2	41	6.9	10.1	8.8 a	(23-) 25—28	9—25	4—11	7.0	B V-1
AG-3	6	8.4	10.1	9.4 a	(20-) 23—25	(6-) 9—17	4—10	6.6	S T-5
AG-4	12	6.2	9.1	7.8 b	25—28 (-30)	(9-) 25—31	2— 6	3.1	G M-4
AG-5	23	6.8	9.7	8.4 c	23—28	11—22	4—15	8.3	B V-4

* Duncan's multiple range test (5% level)

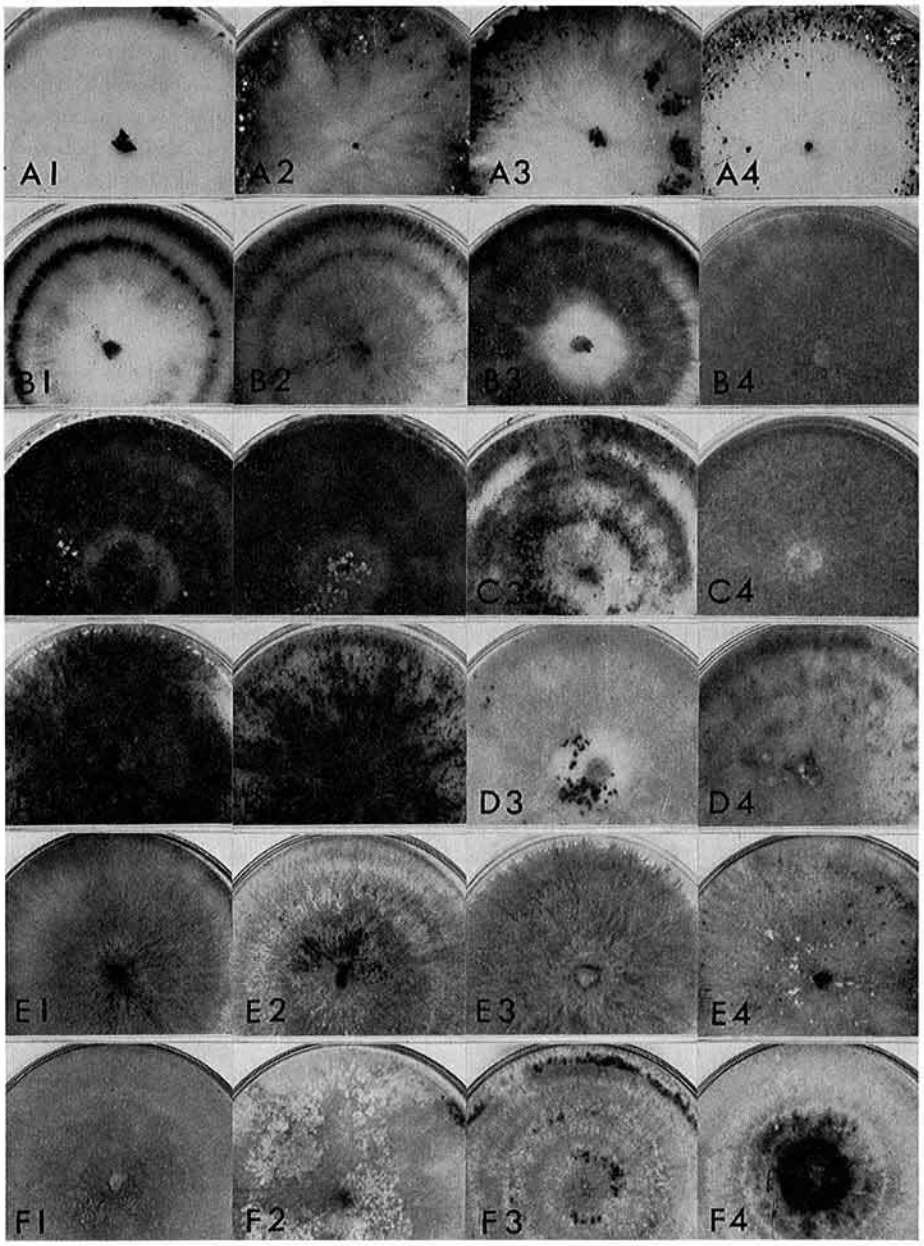


Plate 1. Appearances of mature cultures of representative isolates in five anastomosis groups and two types of *Rhizoctonia solani* grown on PDA at 25°C.

A1–A4: AG–1, B1–B4: AG–2 Type–1, C1–C4: AG–2 Type–2, D1–D4: AG–3, E1–E4: AG–4, F1–F4: AG–5.

narrow next to AG-4. The feature of their cultural appearances was yellowish.

Unassigned isolates. The majority of 13 isolates of unassigned isolates were from sugar beets and soils.

Comparison of anastomosis groups in Japan with groups reported in literatures

Parmeter et al.[6] and Sherwood[9] reported that isolates of *R. solani* were divided into four anastomosis groups. Twelve isolates from these four anastomosis groups were supplied to the author by courtesy of Sherwood. Anastomosis were observed between these 12 isolates and the representative isolates in Japan. Table 4 shows that AG-1, AG-2, AG-3, and AG-4 of Parmeter et al. are identical with AG-1, AG-2, AG-3, and AG-4 of Japan. Moreover, it is suggested that there may be two types in AG-2 of Parmeter et al. as well as AG-2 of Japan.

From the results and evidences in literatures, AG-1, AG-2, AG-3, and AG-4 correspond with I, II, III, and IV of Schultz[8], A, D, F, and C of Richter & Schneider[7]. Moreover, AG-1, AG-2 Type-1, AG-2 Type-2, AG-3, and AG-4 correspond with sasakii type and web-blight type, Winter crop type, rush type and root rot type, potato type, and praticola type of Watanabe & Matsuda[11], respectively. AG-5 perhaps corresponds with B of Richter & Schneider (Table 5).

Conclusion

It is suggested that there may be pathological, ecological, and morphological differenciation in *R. solani*. It is considered that this differenciation can be visualized in anastomosis groups.

It is obvious that *R. solani* in Japan consists of five anastomosis groups from abovementioned results. However, AG-5 has not reported in other studies except the study of Richter & Schneider. AG-2 in Japan is divided into two types, and it is suggested that AG-2 of Parmeter et al. is also divided into two types. Further studies are necessary on these points.

Grouping of *R. solani* with anastomosis is the most easy and useful technique, because

Table 4. Anastomosis between AG-1—AG-4 in Japan and AG-1—AG-4 of Parmeter et al.

Parmeter et al. / Ogoshi		AG-1			AG-2					AG-3	AG-4		
		C-65	S-295	SC-194	ATCC-10159	ATCC-10176	S-280	S-265	S-284	ATCC-6211	S-287	C-283	ATCC-10177
AG-1	R1-2-1	++	++	++									
	R1-2-2	++	++	++									
	A-10	++	++	++									
AG-2 Type-1	FC-1				++	++	−	+	−				
	No. 28				++	++	−	−	+				
	C-1				++	++	−	−	++				
AG-2 Type-2	No. 64				−	−	+	+	++				
	R1-2-6				+	+	+	++	++				
	C-112				+	−	+	++	++				
AG-3	P-11									++			
	P-14									++			
	No. 104									++			
AG-4	AH-3										++	++	++
	GM-7										++	++	++
	RS-6										++	++	++

−, +, ++ Same in Table 2.

Table 5. Comparison of five anastomosis groups of *Rhizoctonia solani* Kühn with the groups in several reports

Ogoshi (1972 b)	Schultz (1936)	Richter & Schneider (1953)	Parmeter et al. (1969)	Watanabe & Matsuda (1966)	
AG-1	I (var. *hortensis*)	A	AG-1	Sasakii type (IA)	*Corticium sasakii*
				Web-blight type (IB)	*C. microsclerotia*
AG-2 Type-1	II (var. *brassicae*)	D (Crucifer group)	AG-2	Winter crop type (II)	
AG-2 Type-2	II	D	AG-2	Rush type (III B)	
				Root rot type (IV)	
AG-3	III (var. *typica*)	E (potato group)	AG-3	Potato type (IV)	
AG-4	IV (var. *cichorii endiviae*)	G	AG-4	Praticola type (III A)	*C. praticola*
AG-5 B		B			

anastomosis groups are not inconsistent with groups that were reported by many workers of *R. solani*.

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