

Rhizoctonia solani

Kuhnの菌糸融合群間におけるビタミン要求の特異性

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Specificity in Vitamin Requirement among Anastomosis Groups of *Rhizoctonia solani* Kühn*

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生越 明**・宇井格生** : *Rhizoctonia solani* Kühn の菌糸融合群
間におけるビタミン要求の特異性

Abstract

The influence of vitamins on the growth of hyphal anastomosis groups of *Rhizoctonia solani* Kühn, AG-1 (IA, sasakii type), AG-1 (IB, web-blight type), AG-2-1 (winter crop type), AG-2-2 (IIIB, rush type), AG-2-2 (IV, root rot type), AG-3 (potato type), AG-4 (praticola type), and AG-5, were studied. All isolates of AG-2-2 (IIIB), AG-2-2 (IV), and AG-5 barely grew in the medium without thiamine hydrochloride, and showed 10 ~ 20-fold growth at 10^{-5} M thiamine, i.e. these groups are thiamine auxotrophic. The stimulating effect of thiamine was slight at 10^{-10} M and obvious at 10^{-9} M, and the mycelial dry weights at 10^{-8} or 10^{-7} M were almost the same at 10^{-5} M. The need for thiamine is a specific character of the group, not of the isolate. Most of AG-2-1, AG-2-2 (IIIB), AG-2-2 (IV) and AG-5 isolates had their growth somewhat stimulated with 10^{-5} M of calcium pantothenate, but were not auxotrophic. There were two isolates of AG-2-1 that might be Ca-pantothenate auxotrophic. Some isolates had stimulated their growth with biotin, folic acid, p-aminobenzoic acid, inositol, nicotinic acid, pyridoxine hydrochloride, and riboflavin, but were not auxotrophic. The need for these vitamins is a character of the isolate, not of the group.

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Introduction

There are some reports about the influence of vitamins on the growth of *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris*)^{1,2,5,7,9,11,12}, and recently Sherwood¹⁰ reviewed on this subject. The differing results of vitamin requirement is attributed to the heterogeneity of the isolates of *R. solani* used by various authors.

Meanwhile, the grouping of *R. solani* with hyphal anastomosis was developed^{4,9}, and the anastomosis groups are now included in studies of *R. solani*. In Japan, eight groups of *R. solani* have been recognized : AG-1 (IA, sasakii type), AG-1 (IB, web-blight type), AG-2-1 (II, winter crop type), AG-2-2 (IIIB, rush type), AG-2-2 (IV, root rot type), AG-3 (IV, potato type), AG-4 (IIIA, praticola type), and AG-5. These are characterized by cultural, pathological, and ecological differences^{4,13}.

It is suggested from the studies by Sherwood⁹ and Ui & Mitsui¹² that the vitamin requirement of *R. solani* isolates is not different for the isolates but is different for the anastomosis groups. The purpose of this study is to investigate vitamin requirements for the growth of *R. solani* anastomosis groups.

* A part of this study was reported at the annual meeting of Phytopathological Society of Japan in 1977.

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Materials and Methods

Isolates. *R. solani* tested were isolates supplied by Dr. Oniki and Mr. Abe, stock cultures in Laboratory of Plant Pathology, Faculty of Agriculture, Hokkaido University, and those used in the previous study by Ogoshi⁴⁾. At least 10 isolates of 8 groups, AG-1 (IA), AG-1 (IB), AG-2-1, AG-2-2 (IIIB), AG-2-2 (IV), AG-3, AG-4 and AG-5, were tested.

Chemicals. Vitamins used were thiamine hydrochloride, D-biotin, calcium pantothenate, pyridoxine hydrochloride, p-aminobenzoic acid, riboflavin, inositol, nicotinic acid, folic acid (Wako Pure Chemical Industries, LTD.). The vitamins were dissolved in 20 % ethanol and stored in a refrigerator.

Media. Potato sucrose agar (PSA) was used for the stock culture of isolates.

The basal medium for testing the influence of vitamins was glucose asparagine medium (GA), having the following composition : 10 g D-glucose ; 2 g L-asparagine ; 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 1 g KH_2PO_4 ; 0.2 mg Fe^{+++} , 0.2 mg Zn^{++} , 0.1 mg Mn^{++} as $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, respectively in 1,000 ml of distilled deionized water, adjusted to pH 6.0 with NaOH. To the medium was added 5 g/l of charcoal, the resulting mixture was then filtered, bottled, and autoclaved.

This medium, solidified with 2 % purified agar, was referred as GAA.

Cultural condition. Isolates tested were previously cultured on PSA and then transferred to GAA. An agar block (7 mm diam.) was cut from the margin of actively growing colony on GAA and floated on the surface of 30 ml GA in 100 ml conical flask into which vitamin had been previously added. The same medium without vitamin was inoculated as control. The inoculated flasks were incubated at 25 C in the dark.

Measurement of growth. After incubation for the experimental period, the mycelial mat was taken out of the flask, dried at 105 ~ 110C, and weighed.

Results

Influence of thiamine hydrochloride—

Concentration of thiamine. The mycelial weight of 2 isolates of each of the 8 groups was measured after 1 or 2 weeks incubation. The concentrations of thiamine tested were 10^{-5} to 10^{-8}M . A part of the results is shown in Table 1. The mycelial weight of the isolates belonging to AG-2-2 (IIIB), AG-2-2 (IV), and AG-5 showed a remarkable increase where there had been an addition of thiamine. The mycelial weights at 10^{-7}M were the same as at 10^{-5} and at 10^{-4}M . The remaining 5 groups showed little difference in growth with thiamine ; that is, there was little or no increase in growth.

The influence of thiamine on the growth of AG-2-2 (IIIB), AG-2-2 (IV), and AG-5 which were found to be thiamine auxotrophic in the preliminary test was investigated at concentrations from 10^{-5} to 10^{-12}M with 10 days incubation. The isolates of these 3 groups showed an increase in mycelial weights related to the concentration of thiamine (Table 2). The stimulating effect of thiamine was obvious at 10^{-9}M but the mycelial weight at 10^{-7}M was almost the same as at 10^{-5}M . The growth of the AG-2-1 isolates used as a control showed little difference from 10^{-7} ~ 10^{-12}M and was almost the same as the control without thiamine.

Incubation periods and thiamine concentrations. The growth of each of the

Table 1. Influence of thiamine concentrations and incubation periods on growth of *Rhizoctonia solani*

Group	Isolate	Incubation period (week)	Mycelial dry weight (mg)					
			Thiamine concentration					
			0	10 ⁻⁸ M	10 ⁻⁷ M	10 ⁻⁶ M	10 ⁻⁵ M	10 ⁻⁴ M
AG-1 (IA)	A-10	1	99	191	180	187	176	
		2	161	170	175	188	173	179
AG-1 (IB)	No. 24	1	165	153	120	126	174	
		2	161	168	169	164	157	158
AG-2-1	PS-4	1	107	114	170	129	143	
		2	181	206	203	197	207	210
AG-2-2 (IIIB)	ZK	1	0	89	137	182	151	
		2	2	142	164	169	173	181
AG-2-2 (IV)	ZK-III	1	8	60	65	111	94	
		2	21	172	190	189	213	210
AG-3	P-11	1	41	88	82	75	85	
		2	174	147	143	178	186	186
AG-4	H-78	1	214	222	231	221	231	
		2	204	192	192	201	207	217
AG-5	GM-10	1	0	94	149	158	184	
		2	86	171	179	187	185	196

Table 2. Influence of thiamine concentrations on growth of AG-2-2 (IIIB), AG-2-2 (IV) and AG-5 isolates

Group	Isolate	Mycelial dry weight (mg)							
		Thiamine concentration							
		0	10 ⁻¹² M	10 ⁻¹¹ M	10 ⁻¹⁰ M	10 ⁻⁹ M	10 ⁻⁸ M	10 ⁻⁷ M	10 ⁻⁵ M
AG-2-2 (IIIB)	C-96	5	5	6	11	31	167	177	185
	C-127	6	6	11	18	29	163	176	189
AG-2-2 (IV)	R-20	4	4	5	9	47	145	165	169
	H-17	3	8	9	9	39	175	187	182
AG-5	GM-10	5	6	5	9	27	136	184	205
	A	7	6	6	8	23	87	177	187
AG-2-1	PS-4	62	77	73	55	63	58	81	125
	F-15	182	184	190	185	180	184	181	182

isolates from 8 groups at 10⁻⁴M thiamine was measured after 1, 2 and 3 weeks incubation. The growth of 4 isolates reached a maximum after 1 week and the other 4 isolates reached a maximum after 2 weeks.

The growth of each of the 2 isolates of AG-2-2 (IIIB), AG-2-2 (IV) and AG-5 was measured after 5, 10, 15, 20 and 25 days incubation with 10⁻⁹, 10⁻⁸ and 10⁻⁵M of thiamine concentrations. The mycelial dry weight reached a near maximum after 10 days (Table 3). The growth increased slightly at 10⁻⁹M and remarkably at 10⁻⁸ and 10⁻⁵M.

Comparison among the groups. The effect of 10⁻⁵M thiamine on the growth of each of the 10 isolates from 8 groups was tested after a period of 10 days incu-

From the above results, the following experiments were performed with an incubation period of 10 days.

Table 3. Influence of thiamine concentrations and incubation periods on growth of AG-2-2 (IIIB), AG-2-2 (IV) and AG-5 isolates

Group	Isolate	Conc. of thiamine (Mol)	Mycelial dry weight (mg)				
			Incubation period (day)				
			5	10	15	20	25
AG-2-2 (IIIB)	C-96	None	8	8	11	11	8
		10^{-9}	28	33	34	30	28
		10^{-8}	104	181	158	157	141
		10^{-5}		158			
	C-330	None	9	6	8	10	7
		10^{-9}	33	35	34	43	35
		10^{-8}	143	193	185	189	184
		10^{-5}		212			
AG-2-2 (IV)	R-20	None	7	6	4	9	5
		10^{-9}	19	32	26	32	25
		10^{-8}	61	154	149	136	126
		10^{-5}		184			
	B-70	None	5	6	5	10	6
		10^{-9}	16	26	27	42	104
		10^{-8}	52	119	139	145	131
		10^{-5}		134			
AG-5	GM-1	None	7	6	8	10	4
		10^{-9}	27	33	26	20	21
		10^{-8}	95	158	166	164	141
		10^{-5}		238			
	A	None	9	9	4	9	8
		10^{-9}	26	26	19	16	20
		10^{-8}	60	93	92	90	118
		10^{-5}		206			

bation. The summary of results is shown in Table 5, All isolates of AG-2-2 (IIIB), AG-2-2 (IV), and AG-5 were thiamine auxotrophic and those of other groups were autotrophic, although here the growth of some isolates, especially of AG-1, was slightly promoted. Similar results were shown after 1 and 2 weeks incubation. Moreover, 19 isolates of AG-2-2 (IV) tested were found to be thiamine auxotrophic.

Influence of D-biotin

The mycelial dry weights of each of 3 isolates from the 8 groups were measured after 10 days incubation at the concentration range of $10^{-6} \sim 10^{-9}$ M of biotin. As all thiamine auxotrophic isolates grew so slightly the effect of biotin could not be decided. When 10^{-5} M thiamine was added, the growth of the isolates was so luxuriant that the effect of biotin, if any, was not detectable.

The effect of biotin was tested on GA containing 10^{-9} M thiamine, and a part of the results is shown in Table 4. Some of the isolates belonging to AG-1 (IA), AG-1 (IB), AG-2-1, and AG-5 were slightly stimulated in their growth, but this

was insufficient to prove biotin auxotrophicity.

Table 4. Influence of D-biotin concentrations on growth of 8 groups of *Rhizoctonia solani*

Group	Isolate	Mycelial dry weight (mg)				
		Concentration of biotin (Mol)				
		0	10^{-9}	10^{-8}	10^{-7}	10^{-6}
AG-1 (IA)	CS-Ka	168	179	190	166	176
AG-1 (IB)	Chiba-2	177	176	173	179	182
AG-2-1	PS-4	164	167	184	182	184
AG-2-2 (IIIB)	C-127	43	50	45	73	75
AG-2-2 (IV)	SA74-17	54	34	37	59	56
AG-3	R-526	179	187	203	215	209
AG-4	ARS-5	183	179	183	196	201
AG-5	R-470	46	42	40	64	65

The effect of 10^{-6} M biotin on each of 10 isolates from the 8 groups was tested, and the results are summarized in Table 5. No isolate was found to be biotin auxotrophic.

The result was that biotin appears to have a minor growth stimulating effect with some difference among the isolates.

Table 5. Influence of vitamins on the growth of anastomosis groups of *Rhizoctonia solani*

		AG-1 (IA)	AG-1 (IB)	AG-2-1	AG-2-2 (IIIB)	AG-2-2 (IV)	AG-3	AG-4	AG-5
Thiamine hydrochloride	10^{-5} M	1.27*	1.26	1.14	16.25	10.41	1.13	1.04	17.53
D-biotin	10^{-6} M	1.05	1.08	1.11	1.01	1.00	0.99	1.00	1.08
Calcium pantothenate	10^{-5} M	1.04	0.98	1.24	1.19	1.28	1.01	1.03	1.39
Riboflavin	10^{-5} M	1.01	1.01	1.20	0.97	0.95	1.01	1.00	1.09
Folic acid	10^{-5} M	1.01	1.05	1.06	1.03	1.07	0.96	1.00	0.97
p-aminobenzoic acid	10^{-5} M	1.01	1.06	0.99	1.01	1.07	0.95	1.01	1.02
Inositol	10^{-5} M	1.02	0.95	1.01	1.03	1.01	1.00	1.01	1.16
Nicotinic acid	10^{-5} M	1.01	1.03	1.02	1.02	1.05	0.95	0.99	1.13
Pyridoxine hydrochloride	10^{-5} M	1.03	0.98	1.01	1.04	0.90	0.99	1.02	1.02

* Ratio of mycelial dry weight grown in vitamin amended medium when the weight in vitamin free medium is regarded as 1.00. The data above are expressed as the means of ten isolates.

Influence of Calcium pantothenate

The effect of 10^{-5} and 10^{-7} M Ca-pantothenate was tested on GA containing 10^{-9} M thiamine. A summary of the results is shown in Table 5. Most of AG-2-1, AG-2-2 (IIIB), AG-2-2 (IV) and AG-5 isolates were somewhat stimulated with 10^{-5} M Ca-pantothenate. The effect was less at 10^{-7} than at 10^{-5} M. Ca^{++} was

not responsible for the above effect, as there was little stimulation of growth on GA containing the same molar concentration using CaCl_2 .

Out of 16 AG-2-1 isolates, two isolates showed 11.7 and 5.1 folds of growth with 10^{-5}M Ca-pantothenate as compared with a control. Both isolates might be Ca-pantothenate auxotrophic.

Influence of riboflavin

The effect of 10^{-5}M riboflavin was tested on GA containing 10^{-9}M thiamine. A summary of the results is shown in Table 5. Eight of 10 AG-2-1 isolates were slightly stimulated. In other groups, some isolates were also slightly stimulated, however, the increase of their mycelial weights was not remarkable.

Influence of other vitamins

Besides the above vitamins, folic acid, p-aminobenzoic acid, inositol, nicotinic acid and pyridoxine hydrochloride were tested with the same procedures. Out of 80 isolates tested, 15 with folic acid, 15 with p-aminobenzoic acid, 18 with inositol, 15 with nicotinic acid, and 15 with pyridoxine increased their mycelial dry weights more than 10 %. The effect of these vitamins differed according to the isolate.

Discussion

In studying the effect of vitamins on the growth of *R. solani*, that of thiamine has been studied most frequently. Elarosi¹⁾, Mathew³⁾, Sadasivan & Subramanian⁸⁾ and Sims¹¹⁾ reported that *R. solani* was thiamine auxotrophic. However, one isolate of Ross⁷⁾ was thiamine auxotrophic, and another was autotrophic. Ui & Mitsui¹²⁾ reported that 25 of 57 isolates increased their mycelial weights and 32 did not with $10\text{ }\mu\text{g/l}$ of thiamine. Sherwood⁹⁾ stated that in AG-2 8 isolates were thiamine auxotrophic, and 4 were not. Our results indicated that the effect of thiamine showed an discrepancy for the groups of *R. solani*; i. e. AG-2-2 (IIIB), AG-2-2 (IV) and AG-5 are thiamine auxotrophic and others are autotrophic. AG-2 is divided into 2 groups, AG-2-1 and AG-2-2, according to the frequency of hyphal anastomosis, and the latter is further divided into 2 subgroups, IIIB and IV, according to physiological characteristics and pathogenicity^{4, 13)}. AG-2-1 is thiamine autotrophic and AG-2-2 is auxotrophic. The evidence by Sherwood⁹⁾ that there are both thiamine auto- and auxotroph in AG-2 indicates the presence of AG-2-1 and AG-2-2. Although AG-5 is thought to correspond to B group of Richter & Schneider⁶⁾, there is no report on this group other than Ogoshi⁴⁾. All isolates of this group are also thiamine auxotrophic.

Mathew³⁾ and Sadasivan & Subramanian⁸⁾ stated that *Corticium microsclerotia* was thiamine auxotrophic. Watanabe & Matsuda¹³⁾ reported that *C. microsclerotia* may be the web-blight type (IB), and this belongs to AG-1⁴⁾. All isolates of AG-1 are autotrophic from the results of the present experiments and Sherwood⁹⁾. A problem arises if *C. microsclerotia* tested by Mathew and Sadasivan & Subramanian was AG-1 of *R. solani*.

Mathew and Sadasivan & Subramanian obtained the highest growth of *R. solani* at 0.1 ppm of thiamine, and Sherwood¹⁰⁾ at 0.02 ppm. In the present result the maximum growth was obtained at 10^{-7}M (ca. 0.03 ppm) and the mycelial weight was almost same at 10^{-7} to 10^{-4}M (30 ppm). Khan & Azam²⁾ and Sadasivan & Subramanian⁸⁾ reported the growth of *R. solani* was inhibited at 1.0 ppm of thiamine. The reason for this difference is unknown.

For vitamins other than thiamine, Khan & Azam²⁾ reported that nicotinic acid,

riboflavin and biotin promoted the growth of *R. solani*. Elarosi¹⁾ also reported the promotion of growth by biotin, inositol, and pyridoxine. On the other hand, testing the effect of biotin on 57 isolates of *R. solani*, Ui & Mitsui¹²⁾ reported that the growth of isolates did not show much difference with or without the addition of biotin. Similar results were obtained in this report, although some isolates were slightly stimulated. The same effect was obtained with folic acid, p-aminobenzoic acid, nicotinic acid, pyridoxine hydrochloride, and riboflavin.

Ca-pantothenate slightly stimulated the growth of AG-2-1, AG-2-2 (IIIB), AG-2-2 (IV), and AG-5 isolates, however, these are not auxotrophic except for two isolates of AG-2-1, which are regarded as variants since the cultural characteristics on agar media are not typical for AG-2-1.

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Literature cited

1. Elarosi, H.M. (1957). Ann. Botany (NS) 28 : 569-585.
2. Khan, M. W. and Azam, M. F. (1975). Indian Phytopath. 28 : 202-205.
3. Mathew, K. T. (1953). Proc. Indian Acad. Sci. (B) 38 : 1-10.
4. Ogoshi, A. (1976). Bull. Natl. Inst. Agric. Sci. Ser. C 30 : 1-63.
5. Parmeter, J. R. Jr., Sherwood, R. T. and Platt, W. D. (1969). Phytopathology 59 : 1270-1278.
6. Richter, H. und Schneider, R. (1953). Phytopath. Z. 20 : 165-226.
7. Ross, D. J. (1960). New Zealand J. Sci. 3 : 219-257.
8. Sadasivan, T. S. and Subramanian, C. V. (1954). Trans. Brit. mycol. Soc. 37 : 426-430.
9. Sherwood, R. T. (1969). Phytopathology 59 : 1924-1929.
10. Sherwood, R. T. (1970). in Parmeter, J. R. Jr. (ed.) Rhizoctonia solani, Biology and Pathology, Univ. California Press, p. 69-92.
11. Sims, A. C. Jr. (1960). Phytopathology 50 : 282-286.
12. Ui, T. and Mitsui, Y. (1960). Ann. Phytopath. Soc. Japan 25 : 63 (abstr.).
13. Watanabe, B. and Matsuda, A. (1966). Designated Experiment (Plant Diseases and Insect Pests) No. 7, Agriculture, Forestry, and Fisheries Research Council and Ibaraki Agric. Exp. Sta. pp. 131.

和 文 摘 要

Rhizoctonia solani Kühn の菌糸融合群間におけるビタミン要求の特異性

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R. solani の AG-1 (IA, イネ紋枯病系), AG-1 (IB, 樹木苗くもの巢病系), AG-2-1 (II, アブラナ科低温系), AG-2-2 (IIIB, イ紋枯病系), AG-2-2 (IV, サトウダイコン根腐病系), AG-3 (IV, ジャガイモ低温系), AG-4 (IIIA, 苗立枯病系), AG-5 の 8 群について, 生育に及ぼすビタミン類の影響を調べた。AG-2-2 (IIIB), AG-2-2 (IV) および AG-5 の全菌株は, thiamine hydrochloride 無添加培地ではほとんど生育せず, thiamine (10^{-5} M) 添加培地では10~20倍の生育量を示し, thiamine 要求性であった。Thiamine は 10^{-10} M で生育促進効果を示し, 10^{-8} , 10^{-7} で, 10^{-5} M とほぼ同等の生育を示した。*R. solani* の thiamine 要求性は菌株よりもむしろ, 菌群の特性であると考えられる。AG-2-1, AG-2-2 (IIIB), AG-2-2 (IV), AG-5 の多くの菌株は Ca-pantothenate (10^{-5} M) で若干の生育促進を示したが, 要求性とはいえない。AG-2-1 の菌株の中には, Ca-pantothenate 要求性とみられる菌株が2株あった。Biotin, folic acid, p-aminobenzoic acid, inositol, nicotinic acid, pyridoxine hydrochloride, riboflavin によって生育促進される菌株があったが, その効果は小で, 要求性とはいえない。これらは菌群間の差ではなく, 菌株間の差であった。