PART II

THE VITAMIN, CARBON AND NITROGEN NUTRITION OF *PIRICULARIA ORYZAE* CAV.

S. Y. Lin, and C. C. Chien[1]

Introduction

The existence of physiologic races of the rice blast fungus has been reported from different rice growing countries. (3, 4, 10, 11, 13, 16, 17, 18). In Taiwan, numerous isolates of *P. oryzae* have been characterized into physiologic races on the basis of their pathogenicity to the differential rice varieties (7, 8, 12, 15).

Isolates of *P. oryzae* also differed in their physiologic characteristics such as mycelial growth, pigmentation, conidium production, and nutritional requirement. Several workers (1, 7, 12, 28) compared the cultural characteristics of a number of the rice blast fungal isolates and classified them into different cultural types. Suzuki (24) analyzed a large number of single spore isolates and grouped them into 11 biologic species on the basis of appressorium type. Chien *et al* (7) found that cultural types of the blast fungus were to some extent associated with pathogenicity, but no definite correlation was observed between cultural characters and pathogenic races.

Many workers have studied the effects of carbohydrates (2, 5, 6, 14, 22, 23, 25, 29, 30), nitrogen sources (2, 5, 6, 14, 20, 21, 23, 25, 27, 29) and vitamins (2, 14, 21, 23) on the growth of *P. oryzae*. Among them, Otsuka in Japan, suggested the close relationship between relative strength of pathogenicity and nutrition of the fungus. He assumed that the difference in pathogenicity shown by races of *P. oryzae* was caused by differences in their metabolical mechanism and biochemical properties, and that the differences in utilization of nutrition were considered to be the result of biochemical differences among the strains, and were available as a basis of classification (23). Chen (6) also stated that the sensitivity of *P. oryzae* to the nutritional status of the host was partly due to its specific nutritional requirements. Others workers (14, 30) reported that nutrition of *P. oryzae* varied in different isolates and this variation was similar to the variation in virulence of the isolates to different rice varieties

The primary purpose of the investigations was to study the vitamin, carbon and nitrogen nutrition of *P. oryzae* and to elucidate the possible correlation between nutrition and pathogenicity of the fungus.

---

1. Associate Plant Pathologists, Department of Plant Pathology, Taiwan Agricultural Research Institute, Taipei, Taiwan, China.

Meterials and Methods

Isolates of *P. oryzae* showing distinct degree of pathogenicity were used. They included ten Taiwan isolates, one Japanese and one U.S. isolate.

The basal medium consisted of KNO_3 2 g, KH_2PO_4 0.5 g, K_2HPO_4 0.5g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCl_2 \cdot 6H_2O$ 0.1 g, $FeSO_4 \cdot 7H_2O$ 0.0075 g, $CuSO_4 \cdot 5H_2O$ 0.007 g, $MnSO_4 \cdot 6H_2O$ 0.002 g, $ZnSO_4 \cdot 7H_2O$ 0.009 g, H_3BO_3 0.0009 g, dextrose 30 g, and distilled water to make up one liter. When the effect of carbon or nitrogen compound on the growth of the fungus was studied, 2 mg. thiamine and 5 μg biotin were added to one liter of the basal medium.

Vitamins were added to give the final concentration indicated: thiamine 2 mg, biotin 0.005 mg, riboflavin 0.5 mg, pyridoxine hydrochloride 0.5 mg, calcium pantothenate 2 mg, p-aminobenzoic acid 0.5 mg, inositol 4 mg, nicotinic acid 5.0 mg, folic acid 0.004 mg, choline chloride 2 mg, and ascorbic acid 0.5 mg in one liter medium. To determine optimum concentration of thiamine and biotin for growth, concentrations listed in Table 4, and 5 were utilized.

The carbon or nitroge sourses were prepared to yield the amount of carbon equivalent to that in 30 g of glucose, and the nitrogen compound equivalent to that in 2 g potassium nitrate per liter of medium.

All media were sterilized by Seitze filtration, unless otherwise stated. In preliminary experiments, the solid media were sterilized at 100°C for 30 minutes thrice at 24 hours interval. The pH of media was measured by Leeds pH meter and adjusted to pH 7.0 by adding 1.0 N NaOH. The pH values of the residual liquid media was determined at the end of all experiments.

The fungus was grown in 30 ml of liquid medium contained in 125 ml Erlenmeyer flask. Some experiments were conducted in solid media, which were prepared by adding 2% Difco Bacto-agar and distributed in square flasks.

Inoculum used was a mycelial disc cut from the edge of a 14-day-old colony on the vitamine-free basal medium with a 5 mm cork borer. Stationary cultures were utilized in all experiments. Samples on liquid media were harvested after 20 days incubation at 28°C. The compact mycelial mat was washed several times with distilled water. A water suction pump was used to speed the harvest. The mycelial mat from the quintuplicates were collected respectively. After drying the mycelial mat at 60°C for 24 hours on previously dried and weighed filter paper, the dry weight of mycelium was determined. The results are presented as the average weights of the five replicates in mg dry weight.

To determine spore production on solid media, ten 5 mm diameter mycelial discs were cut from each colony after 7 and 14 days incubation at 28°C, and transferred into a Kahn tube containing one ml distilled water. The mycelial discs in the tube were homogenized and shaken vigorously and spore count was made with a hemocytometer.

Results

Effect of vitamins: In initial experiments to determine which among the 11 vitamins was responsible for the growth and sporulation of the fungus, the media were prepared so

that each of the 11 vitamins was withheld in turn, the other 10 vitamins being present. There are two controls, one the basal medium only, and the other the basal medium containing all 11 vitamins. The results are given in Table 1. Among the 11 vitamins used only biotin and thiamine were influential to the growth of the fungus. Other vitamins were found ineffective. The dependency of the fungus on biotin was greater than on thiamine. In the biotin free medium, most of the isolates showed very slight mycelial growth. Some isolates such as U.S. 5A, 4S-26S, and 4T-28S required much more exogenous biotin than other isolates. They gave almost no growth on biotin-free medium. The dependency of the fungus on thiamine varied from almost a complete requirement to partial requirement depending on the isolates. It was found that a majority of the isolates had a partial requirement for thiamine. Some isolates appeared to have almost no thiamine requirement, whereas most of the other isolates showed varying degrees of deficiency for thiamine. However, no isolate exhibited an absolute requirement for this vitamin. In the thiamine-free medium, isolates 4S-26S and 4C-10S produced as much mycelium as that in "all" medium. Three isolates i.e., 4S-15S, 3C-58G,and 4K-4S produced 1/3-1/2 of mycelial dry weight, and the other four isolates, 0'S-45S, 4T-28S, Ken 60-19 (C-1), and U.S. 5A gave 2/3 growth as compared with that in "all" medium.

In general, isolates of *P. oryzae* in the media devoid of vitamins other than biotin or thiamine produced the dry weight of mycelium as much as in "all" medium. Some isolates, such as isolate 3C-58G for example, produced more dry weight of mycelium in media devoid of ascorbic acid or p-aminobenzoic acid. From these results, it was apparent that none of the fungal isolates showed a multiple vitamin requirement with regard to the 11 vitamins used in this study. Only biotin and thiamine, especially the former, were found to be indispensable to the growth of the fungus.

Sporulation of the fungus varied greatly with isolates and with media. Generally, spores were produced much more abundantly after 14 days incubation. In some instances more spores were produced in the medium devoid of a certain vitamin than in "all" medium. Biotin and thiamine also greatly influenced the sporulation of the fungal isolates. No conidium was produced by any isolates in either biotin or thiamine-free media. It was demonstrated that both biotin and thiamine were absolutely essential for the sporulation of *P. oryzae*. (Table 2)

Table 1. Growth of *P. oryzae* in a basal medium supplemented with different vitamins and glutamic acid.

Vitamin	Isolate									Treatment mean
	(1) 3C-58G	(12) 4S-15S	(13) O'S-45S	(16) 4S-26S	(20) 4C-10S	(22) 4T-28S	(24) 4K-4S	Ken 60-19 (C-1)	U. S. 5A	
All[a]	137[c]	205	144	158	154	150	171	130	122	152.3
All−thiamine	61	68	100	142	118	92	88	89	85	93.7
All−biotin	32	35	35	15	38	19	30	34	3	26.8
All−inositol	173	197	145	178	156	135	180	132	141	159.7
All−riboflavin	163	158	139	175	129	164	175	154	140	155.2
All−calcium pantothenate	179	186	157	156	161	146	183	156	147	163.4
All−ascorbic acid	183	164	142	142	156	157	155	145	131	152.8
All−nicotinic acid	150	200	144	153	181	143	175	143	124	157
All−folic acid	173	206	125	129	147	154	175	162	117	154.2
All−p−aminebenzoic acid	183	219	129	150	184	125	177	143	120	158.9
All−pyridoxine hydrochloride	156	194	134	145	114	141	171	113	126	143.8
All−choline chloride	164	174	137	98	165	144	161	126	138	145.2
Basal media only	13	36	5	2	27	8	41	21	5	17.6
B.M.[b]+thiamine+biotin	139	296	131	171	157	160	181	137	138	167.8
B.M.+thiamine+biotin+glutamic acid	170	200	131	103	181	152	200	160	131	158.7
Isolste mean	138.4	169.2	119.9	127.8	137.9	126	150.9	123	111.2	

a Basal medium plus 11 vitamins.
b The basal medium.
c Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments=19.2
0.01 between treatments=25.5
L.S.D. 0.05 between isolates=21.8
0.01 between isolates=30.4

Table 2. Sporulation of *P. oryzae* in a basal medium supplemented with different vitamins and glutamic acid.

Vitamin	Isolate																Treatment mean	
	(1) 3C-58G		(12) 4S-15S		(16) 4S-26S		(20) 4C-10S		(22) 4T-28S		(24) 4K-4S		(26) 5K-33S		U.S. 5A			
All[a]	0.8[b]	2.4[c]	2.3	3.5	31.2	78.7	2.2	4.3	26	12.8	0.8	14.7	1.9	9.3	26.1	113.5	11.41	29.9
All−thiamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
All−biotin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
All−inositol	0.9	1.5	5.1	7.0	27.6	33.8	2.8	9.1	51.3	30	1.6	21.6	3	3.4	13.6	132	13.24	31.05
All−riboflavin	2.2	1.2	6.2	8.2	50.7	30.6	5.5	2.2	28.5	18.8	2	16.1	0.8	5.3	35.1	139.1	16.38	27.69
All−calcium pantothenate	1.2	2.1	4.3	4.5	30.0	27.3	2.2	1.5	37.6	15.6	2.8	11.8	1.9	4.3	16	105.4	12	21.56
All−ascorbic acid	0.5	1.6	9.5	8.3	16.4	114.3	2.1	3.8	24.8	23.9	1.5	32	0.7	11.9	35.5	140.9	11.38	32.59
All−nicotinic acid	1.2	2.5	5.6	6.4	43.2	24.1	4.9	3.3	21.8	20.1	0.5	19.8	0.5	4.6	19.7	151.7	12.18	29.06
All−folic acid	2.3	1.5	4.5	6.4	49.8	17.1	1.3	1	28.5	35.8	3.6	24.1	2.5	9.4	82.7	138.7	21.9	39.25
All−p−aminobenzoic acid	0.9	2.3	5.2	5.5	45.7	88.3	2.5	5.2	35.5	32.4	1.7	15.7	1.6	2.8	23.8	109.5	14.61	32.71
All−pyridoxine	0.8	2.4	7.2	8.2	27.8	43.6	5.3	8.8	33.3	19.5	1.9	20.7	3.0	5.6	15.3	114.4	11.83	27.9
All−choline chloride	1.4	1.5	5.5	6.0	29.7	25.8	3	4.9	37.1	34	5.7	16.3	2.3	3.2	6	76	11.34	20.96
B.M. only	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B.M.+thiamine+biotin	0.9	2.2	4.8	5.0	14.9	44.2	4.8	7.4	27	32	2.4	15.5	0.8	8.5	4.9	11.8	7.56	15.83
B.M.+thiamine+biotin+glutamic acid	0.6	1.1	9.5	12.2	15.7	6.3	5.5	16.3	13.6	49.2	0.6	7.3	0.4	17.5	17.3	121.9	9.03	28.98
Isolate mean	0.9	1.5	4.7	5.4	25.5	35.6	2.8	4.5	24.3	21.6	1.7	14.4	1.9	6.4	19.7	90.3		

a Basal medium plus 11 vitamins.
b Number presented in 20 thousands of conidia produced on ten 5mm diameter agar discs after one week incubation.
c Number of conidia produced after two weeks incubation.
One week incubation:
L.S.D 0.05 between treatments=35.18
0.01 between treatments=46.79
no significant differences between isolates
Two weeks incubation:
L.S.D 0.05 between treatments=11.26
0.01 between treatments=34.84
L.S.D. 0.05 between isolates=13.21
0.01 between isolates=43.47

In subsequent experiments, vitamin was individually added to the basal medium which contained thiamine and biotin. All isolates gave 1/2—2/3 mycelial growth in the medium with biotin alone, as compared with those in the medium containing both thiamine and biotin. In the medium devoid of biotin, isolates 4T-28S and U.S. 5A showed almost no growth, the rest of the isolates, gave only slight growth. Addition of any other vitamin in the medium containing biotin and thiamine did not further increase the fungal growth. This fact clearly indicated that no appreciable growth was secured with a single vitamin other than biotin and thiamin. When biotin was used in association with thiamine, the fungal growth was greatly increased. (Table 3).

It may be concluded that biotin was indispensable to the growth of the fungus. Thiamine was only a complimentary growth factor. In the absence of thiamine, several isolates of the fungus were able to make fairly abundant mycelial growth but failed to produce spores. Thus, thiamine was more important for sporulation than for vegetable growth. Thiamine alone could exert certain degree of mycelial growth in some isolates, yet thiamine effected fungal growth and especially sporulation only when a sufficient amount of biotin was present in the medium.

Leaver *et al* (19) reported that both thiamine and biotin were essential for growth of the fungus and that the optimum concentration of biotin was 1.0-10 μg/1. Otani (21) stated that biotin was essential for the growth of *P. oryzae*, its optimum concentration being 2.3-3.0 μg/1. In our studies, the minimum concentration of biotin for the optimum growth of *P. oryzae* was 0.5 μg/1, and of thiamine was 0.02 mg/1. Below these concentrations, the growth of the fungus was drastically reduced. (Table 4, Table 5). It was noted that different isolates of the fungus required different amounts of the same vitamin for optimum growth, however, no significant difference in the growth of a given isolate was observed

Table 3. Growth of *P. oryzae* in a basal medium supplemented with biotin, thiamine and other vitamins

Vitamin	Isolate						Vitamin mean
	(1) 3C-58G	(13) 0'S-45S	(22) 4T-28S	(24) 4K-4S	(26) 5K-33S	U.S. 5A	
B.M.[a]	15[c]	10	5	14	20	8	12
B.M.+biotin	65	102	100	88	77	94	87.7
B.M.+thiamine	28	24	8	27	16	5	18
B.M.+B.T.[b]	188	159	197	187	182	177	181.7
a+b+inositol	171	157	180	191	200	185	180.7
a+b+riboflavin	131	158	194	177	180	188	171.3
a+b+calcium pantothenate	196	142	171	178	176	171	172.3
a+b+ascorbic acid	160	159	185	205	215	156	180
a+b+nicotinic acid	156	167	201	197	188	166	179.2
a+b+folic acid	165	165	184	226	186	153	179.8

a+b+P-aminobenzoic acid	170	188	186	164	190	187	180.8
a+b+pyridoxine hydrochloride	187	145	176	166	179	182	172.5
a+b+cholinc chloride	187	195	186	150	188	150	176
Isolate mean	139.9	136.2	151.8	151.5	153.6	140.2	

a. The basal medium
b. Biotin and thiamine
c. Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments=23.70
0.01 between treatments=31.52
L.S.D. 0.05 between isolates =29.38
0.01 between isolates =42.11

Table 4. The influence of biotin concentration on the growth of *P. oryzae*.

concentration (μg/l.)	(1) 3C-58G	(13) 0'S-43S	(22) 4T-28S	U.S. 5A	Biotin concentration mean
5,000	218[a]	220	210	142	197.5
500	264	220	215	138	209.3
50	200	216	214	128	189.5
5	239	215	195	126	193.8
0.5	125	160	207	144	159.0
0.05	45	52	42	30	42.3
0.005	45	47	25	39	39.0
0.0005	48	45	20	30	35.8
Isolate mean	148	146.9	141	97.3	

a. Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments=39.67
0.01 between treatments=53.97
L.S.D. 0.05 between isolates =48.91
0.01 between isolates =72.01

Table 5. The influence of thiamine concentration on the growth of *P. oryzae*.

concentration (mg/l.)	(1) 3C-58G	(13) 2C-2124S	(22) 4T-28S	U.S. 5A	Thiamine concentration mean
20	220[a]	285	195	186	221.5
2.0	213	310	190	213	231.5
0.2	143	256	185	147	182.8
0.02	95	200	95	105	123.8
0.002	84	120	95	72	92.8
0.0002	56	65	92	62	68.8
Isolate mean	135.2	206	142	130.8	

a. Dry weight of mycelium in mg.
L.S.D. 0.01 between treatments=39.49
0.01 between treatments=54.69
L.S.D 0.05 between isolates =44.80
0.01 between isolates =65.00

with either biotin or thiamine in concentrations above minimum requirement. The minimum requirement of biotin for isolates 3C-58G and 0'S-45S was 5 μg/1, and for isolates 4T-28S and U.S. 5A, 0.5 μg/1. (Table 4). The minimum requirement of thiamine for isolate 3C-58G was 2 mg/1, for isolates 4T-28S and U.S. 5A, 0.2 mg/1, and for isolate 2C-2124S, 0.02 mg/1 (Table 5.).

Effect of carbon sources: The results of carbon nutrition of *P. oryzae* are given in Table 6. The ability of most of the isolates to utilize certain carbon compounds were relatively similar in many instances. Most of the isolates well utilized glucose, sucrose, fructose, starch, maltose, and lactose, and the variation in utilization of these compounds among the isolates was not great. The ability to utilize galactose, dextrin, and glycerol, however, differed with isolates. Galactose was a poor carbon source for most of the isolates, yet its utilization by isolate 3C-58G and 4C-26S was satisfactory. Sorbose was also unsuitable for the fungus growth. Raffinose supported only moderate growth of the fungus. Among polysaccharides tested, only starch was a suitable carbon source for all isolates, while inulin and cellulose were not Dextrin was utilized moderately by all isolates. Alcohols and organic acids were the poor sources of carbon. It was of interest to note that isolate 6C-34S utilized cellulose and glycerol far better than the rest of the isolates. These two compounds were poorly utilized by the rest of the isolates. Rather fluctuating results were shown when xylose, lactose and starch were used as carbon sources. There were no apparent differences in the utilization of single carbon source by the ten isolates. Differences in the growth of a single isolate were recorded, however, when it was grown on different carbon sources.

Effects of nitrogen sources: In the present studies, various nitrogen compounds were utilized to varying extent by 10 isolates of the fungus (Table 7). Among the inorganic nitrogen compounds, nitrate was utilized very well by all isolates, even nitrite was shown as a good nitrogen scurce for the fungus growth. On the other hand, poor growth of the fungal isolates occurred in the ammonium form of nitrogen sources. Suitability of nitrate-N over ammonium-N was reported by Tanaka *et al.* (27), who stated that the inferiority of ammonium-N was caused by the fall of pH in the medium. In our studies, it was the only exception that isolate 5K- 33S produced large amount of mycelium when NH_4Cl was used as the nitrogen source. Urea was also a suitable nitrogen source. All isolates, except 5K-33S, showed good growth in the medium containing urea. Among amino acid tested, glutamine, glutamic acid, asparagine, aspartic acid, leucine, glycine, and serine were well utilized by all isolates. Most isolates utilized alanine and proline to some extent, while poor utilization was shown by 2K-27S in alanine and by 3C-58G in both amino acid media. Utilization of histidine, valine, arginine and methionine, varied with isolates. Tryptophane was utilized well by all isolates with the exception of 4T-28S. Cystein was the most unsuitable nitrogen source for the growth of all isolates. Peptone and casein hydrolysate were also found to be the suitable nitrogen sources. There were no apparent differences among the isolates when the utilization of nitrogen compounds were considered. There were, however, notable differences in yield of mycelium obtained with the different nitrogen compounds for a given isolate.

Table 6. Utilization of carbon sources by 10 isolates of *P. oryzae*

Carbon sources	Isolate										Carbon source mean
	(1) 3C-58G	(3) 2K-27S	(12) 4S-15S	(13) 0'S-45S	(16) 4S-26S	(22) 4T-28S	(24) 4K-4S	(26) 5K-33S	6C-34S	U.S. 5A	
Glucose	203[c]	175	210	215	167	149	184	169	189	200	186.1
Fructose	170	181	187	142	194	173	204	193	181	222	184.7
Galactose	160	29	43	59	120	22	55	37	35	36	59.6
Sorbose	50	30	29	42	47	62	30	36	24	34	33.4
Xylose	125	112	108	79	114	155	150	164	210	205	142.2
Sucrose	228	189	248	210	200	159	195	214	200	213	205.6
Maltose	160	172	151	125	227	137	172	190	194	148	167.6
Lactose	178	155	114	156	108	99	84	105	143	107	124.9
Raffinose	90	64	80	70	68	93	94	100	61	87	80.7
Starch	263	134	216	256	178	166	195	127	153	123	181.1
Dextrin	105	95	105	116	80	95	65	95	93	92	94.1
Inuline	30	28	41	47	53	42	33	38	28	52	40.1
Cellulose[a]	8	10	35	43	35	22	21	6	72	26	27.8
Mannitol	45	40	85	20	40	27	23	42	28	92	44.2
Glycerol	25	40	40	13	34	74	27	40	123	78	49.4
Na-Succinate	95	50	35	37	56	29	30	39	45	33	44.9
Na-Citrate	79	40	31	35	60	24	25	50	21	19	38.4
Control[b]	13	5	10	14	20	8	15	10	12	8	11.5
Isolate mean	112	86.7	99.3	95.3	101.4	85.3	90.2	91.9	102.3	98.6	

a. The medium was sterilized at 100° C for 30 minutes thrice at 24 hours interval.
b. Control medium contains no carbon compound.
c. Dry weight of mycelium in mg.
L.S.D. 0.05 between treatments = 24.92
0.01 between treatments = 32.99
L.S.D. 0.05 between isolates = 28.27
0.01 between isolates = 39.37

Table 7. Utilization of nitrogen sources by 10 isolates of *P. oryzae*

Nitrogen source	Isolate										Nitrogen source mean
	(1) 3C-58G	(3) 2K-27S	(12) 4S-15S	(13) 0'S-45S	(16) 4S-26S	(22) 4T-28S	(24) 4K-4S	(26) 5K-33S	6C-34S	U.S. 5A	
KNO_3	200[b]	210	212	234	183	157	110	110	195	212	183.2
$NaNO_3$	182	202	206	247	177	175	140	133	165	210	183.7
$NaNO_2$	195	202	187	133	236	160	152	207	169	192	183.3
NH_4Cl	65	50	70	47	45	30	64	148	29	30	57.8
NH_4NO_3	60	50	84	53	40	38	60	62	50	48	54.5
$(NH_4)_2SO_4$	50	45	96	65	78	70	60	52	51	81	64.8
Urea	110	120	174	141	140	151	117	58	178	190	137.9
Glutamic acid	226	240	234	215	194	166	124	116	155	225	189.5
Glutamine	171	190	165	242	187	276	168	161	173	236	196.9
Aspartic acid	181	197	240	280	212	280	149	196	187	278	220.0
Asparagine	203	222	201	270	180	174	114	205	200	254	202.3
Histidine	123	111	94	85	57	80	64	75	79	159	92.7
Valine	76	50	111	203	95	94	163	93	60	86	103.1
Alanine	75	89	236	218	112	188	147	201	235	176	167.7
Proline	85	285	181	158	158	222	188	268	173	236	195.4
Leucine	185	203	190	162	108	190	176	235	190	273	191.2
Glycine	240	219	160	156	213	200	173	240	123	232	195.6
Arginine	86	50	152	142	136	180	62	100	70	98	107.6
Methionine	60	52	180	151	80	158	80	186	100	102	114.9
Tryptophane	165	126	171	175	108	85	175	138	192	153	148.8
Serine	188	123	157	220	201	189	178	236	173	212	187.7
Cystein	65	55	77	86	40	23	21	32	30	25	45.4
Casein hydrolysate	176	134	182	196	160	140	154	158	163	120	158.3
Peptone	160	153	189	226	125	106	169	130	164	180	160.2
Control[a]	42	14	18	38	38	21	20	38	25	18	27.2
Isolate mean	138.3	139.3	158.7	165.7	132.1	142.1	121.1	143.1	133.2	161.0	

a. The control medium contains no nitrogen compound.

b. Dry weight of mycelium in mg.

L.S.D. 0.05 between treatments = 25.75

0.01 between treatments = 30.83

L.C.D. 0.05 between isolates = 31.49

0.01 between isolates = 40.10

Discussion

Studies on vitamin nutrition of *P. oryzae* showed that among the eleven vitamins tested only biotin and thiamine were influencial to the growth and sporulation of the fungus. The fungus seemed to have no requirement for the other vitamins. None of the fungal isolates showed a multiple vitamin requirement with regard to the 11 vitamins used in this study. It was found that dry weights of mycelia produced by the isolates in the biotin-free medium and in the basal medium were almost the same, while more mycelia was produced in the thiamin-free medium. The results that isolates of *P. oryzae* required biotin as an indispensable growth factor, and thiamine as a complimentary growth factor coincided with the results obtained by the other workers (21, 23, 26). Most isolates absolutely required an exogenous supply of biotin. Some isolates had only a negligible requirement for thiamine. Different degree of deficiency for biotin or thiamine may reflect different rates of synthesis of these vitamines in the fungal mycelium. Cochrane (9) stated that fungi not requiring or requiring only a negligible amount of exogenous supply of a vitamin must synthesize the compound and that partial deficiencies for a vitamin are probably more common than absolute or complete deficiencies. When fungi synthesize more of a vitamin that they require for optimum growth, the excess may accumulate in the mycelium or excrete into the culture medium (23). Some isolates produced significantly more mycelium in certain vitamin-free medium than in "all" medium. This might be due to the suppressing factor of the vitamin on the growth of the fungus. It needs further study to clarify whether or not these suppressing effects are present.

From the results of sporulation test, it was clearly shown that biotin and thiamine are absolutely essential for the sporulation of *P. oryzae*. In the absence of these two vitamins, several isolates were able to make some mycelial growth, but failed to produce spores. Apparao (2) also found that larger quantities of thiamine and biotin were required for sporulation than for growth. Sporulation of the fungus varied greatly with isolates and with media, yet, to what degree the vitamins other than thiamine and biotin influenced the sporulation of the fungus is not clear.

Otsuka (23) indicated that the requirement of biotin, thiamine and nicotinic acid of strains of *P. oryzae* was closely related to the relative strength of pathogenicity. According to him the majority of isolates which grew only in medium containing both biotin and thiamine were considered to have medium or strong pathogenicity. A few isolates which grew in media devoid of either biotin or thiamine had weak or no pathogenicity. Isolates with strong pathogenicity generally grew in the medium containing biotin and nicotinic acids. In the present study, because of limited number of test isolates, it seemed too early to draw the conclusion. Nevertheless, from our results, it could be inferred that no clear correlation was observed between the pathogenicity and vitamin requirement of the rice blast fungus.

Many workers have studied the carbon nutrition of *P. oryzae*. In general, organic acids

were not suitable carbon sources for the fungus (14, 22, 30). The same results were obtained in our studies. Otani (22) however, reported that glucose, inulin and mannitol, as well as organic acid such as succinic acid are the most excellent carbon sources for the fungus growth. Inulin and mannitol were found to be a poor carbon sources by the present writers. Glycerol was regarded as an unsuitable nutrient by Tomizawa (29), but was reported as an excellent carbon source by Tanaka (25). In our studies, it was noted that most isolates were unable to utilize glycerol, with the exception of isolate 6S-34S which utilized glycerol quite well. According to Otani (22), lactose was unfavorable carbon source. Other workers, however, reported that lactose was well utilized by the fungus (5, 6, 14, 23). Xylose was reported as a suitable carbon source by several workers (14, 22). It was also found to be a relatively poor source of carbon (30). In our studies, all isolates, except 0'S-45S, utilized xylose to a certain extent. Otsuka (23) indicated that there were marked differences among strains in utilization of sorbose, inulin and mannitol. According to him the strongly pathogenic group were inulin, cellulose utilization positive and sorbose utilization negative, and those strain which showed weak pathogenicity belonged to inulin, cellulose utilization negative, sorbose utilization positive group. Chen (5) also reported that isolates showed higher ability in cellulose utilization gave higher pathogenicity. In our studies, there were no apparent differences in the utilization of these carbon sources by the isolates, although utilization of cellulose somewhat differed with isolates.

The blast fungus utilized a wide variety of inorganic and organic nitrogen compounds. In the present study, nitrate and nitrite nitrogen was superior to ammonium nitrogen. $NaNO_2$ was found to be unfavorable for the growth of the fungus (5, 20, 21). Otsuka (23) reported that some strains of the blast fungus grew well in a medium containing $NaNO_2$, while others did not. He stated that strains that did not utilize $NaNO_2$ or utilize it poorly, mostly showed strong pathogenicity, while those strains that utilized it well showed weak pathogenicity. In the present studies, all isolates tested utilized $NaNO_2$ satisfactory, and the difference among isolates was not apparent. Many workers (14, 20, 21) reported that ammonium-N was unsuitable nitrogen source. Tanaka (27) ascertained that the inferiority of ammonium-N was caused by the fall of pH value in the medium. By contrast, Chen (6) stated that ammonium salts were more favorable inorganic N sources than nitrate. Among amino acid tested, glutamine, glutamic acid, asparagine, aspartic acid, leucine, glycine, and serine were well utilized by all isolates. These results are similar to those reported elsewhere (5, 14, 20, 23). Generally, the leaves of susceptible rice plant have higher content of amino acids (25). This may suggeste a possible relationship between the growth responses of *P. oryzae* in culture to amino acids and the establishment of the fungus in the host. It was pointed out by Otsuka (23) that strain of strong pathogenicity generally utilized trytophane poorly, while those of weak pathogenicity generally utilized it well. In our studies, however, no difference in tryptophane utilization was observed among isolates of different pathogenicity. Most isolates utilized tryptophane well, with the exception of isolate 4T-28S. No clear correlation was found between utilization of nitrogen or carbon compounds and the virulence of the isolates.

Summary

Vitamin requirement for growth and sporulation of a certain isolates of *Piricularia oryzae* Cav. were determined. Of the vitamins studied, only biotin and thiamine were essential to the growth of the fungus. Other vitamins were found ineffective. The dependency of the fungus on biotin was greater than on thiamine. All isolates tested required exogenous biotin for mycelial growth. It was found that a majority of the isolates had a partial requirement for thiamine. No isolates exhibited an absolute requirement for thiamine. Both biotin and thiamine were absolutely essential for the sporulation of *P. oryzae*. No conidia were produced by any isolate in either biotin or thiamine free media. In the absence of thiamine, several isolates of the fungus were able to make fairly abundant mycelial growth, but failed to produce spores. The minimum concentration of biotin for the optimum mycelial growth of the fungal isolates was 0.5 μg/1, and of thiamine, 0.02 mg/1. Below these concentrations, the growth of the fungus was drastically reduced. No correlation was observed between pathogenicity and vitamin requirement of the fungus.

The effects of different carbon and nitrogen compounds upon the growth of the fungus were also studied. The best carbon sources for all isolates were glucose, sucrose, fructose, starch, maltose, lactose and xylose. Raffinose was utilized moderately by all isolates. Galactose and Sorbose were found to be unfavorable for most of the test isolates. Alcohols and organic acids were the poor sources of carbon. With the exception of starch, all polysaccharides studied were unfavorable for the fungus growth. Utilization of cellulose by isolates 6C-34S, 0'S-45S and 4C-15S was somewhat better than the other isolates. Dextrin supported only moderate growth of the fungus. Among the inorgainc nitrogen tested, nitrate and nitrite nitrogen were superior to ammonium. Several amino acids such as glutamine, glutamic acid, asparagine, aspartic acid, leucine, glycine, and serine were well utilized by all isolates. Cystein was the most unfavorable amino acid tested. Tryptophane was utilized well by all isolates with the exception of 4T-28S. Most isolates utilized alanine and proline to some extent. Utilization of histidine, valine, arginine and methionine varied with isolates. There were no apparent differences among the isolates when the utilization of nitrogen and carbon sources were considered.

Literature Cited

1. Aoki, Y. 1935. On the physiological specialization in the rice blast fungus, *Piricularia oryzae* Cav. Ann. Phytopath. Soc. Japan 5: 107-120.
2. Apparao, A. 1962. Nutritional factors governing Sporulation of *Piricularia oryzae* in culture. J. Indian Bot. Soc. 41:604-622. (Abstr. in Rev. Appl. Mycol. 43:17).
3. Atkins, J. G. 1962. Prevalence and distribution of pathogenic races of *Piricularia oryzae* in the U.S. Phytopathology 52: 2. (Abstr.)
4. Atkins, J. G. 1965. Physiologic races of *Piricularia oryzae* in the Western Hemisphere, 9. 243-244 In the Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst.,

July 1963. Johns Hopkins Press, Baltimore.

5. Chen. M. J. 1966. The carbon and nitrogen utilization ability of *Piricularia oryzae* Cav. in relation to variation of the pathogenicity. Plant Protection Bulletin (Taiwan) 8 (4): 1-11.
6. Chen, Y. S., H. C. Ren, and C. T. Fang. 1964. Study on the carbon and nitrogen nutrition of *Piricularia oryzae* and *Helminthosporium oryzae*. Acta phytopath. Sin. 7: 165-174. (Abstr. in Rev. Appl. Mycol. 44: 395)
7. Chien, C. C., S. Y. Lin, and S. C. Jong. 1963. Studies on the physiologic races of *Piricularia oryzae* Cav. (II). Agr. Res. (Taiwan) 12: 29-39.
8. Chiu, R. J., C. C. Chien, and S. Y. Lin. 1965. Physiologic races of *Piricularia oryzae* in Taiwan, p. 245-255. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
9. Cochrane, V. W. 1957. Physiolgy of Fungi. John Wiley and Sons, Now York. 246 p.
10. Goto, K. 1960. Progress report of the joint research on the races of the rice blast fungus, *P. oryzae*. Fasc. I, Plant Disease and Insect Pests Forecasting Service Spec. Bull. 5. 89 p.
11. Goto, K. 1965. Physiologic races of *Piricularia oryzae* in Japan, p, 237-242. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
12. Hung, C. S., C. C. Chien and S. Y. Lin. 1961. Study on the physiologic races of *Piricularia oryzae* Cav. Agri. Res. (Taiwan), 10(1): 27-34.
13. International Rice Research Institute. 1964. Study on the pathogenic races of *Piricularia oryzae* Cav. in the Philippines. p. 131-136. In 1964 Annual Report of the International Rice Research Institute, Los Banos, Laguna, Philippines.
14. International Rice Research Institute. 1964. Nutrition of *Piricularia oryzae* isolates in vitro. p. 136-140. In 1964 Annual Report of the International Rice Research Institute.
15. Kou, T. T., S. C. Woo, and W. H. Wang. 1963. Some physiologic specializations of *Piricularia oryzae* Cav. in Taiwan. Bot. Bull. Acad. Sinica 4: 23-29.
16. Latterell, F. M., M. A. Marchetti, and B. R. Grove. 1954. Co-ordination of effort to establish an international system for race identification in *Piricularia oryzae*, p. 257-274. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
17. Latterell, F. M., E. C. Tullis and J. W. Collier. 1960. Physiologic races of *Piricularia oryzae* Cav. Plant Dis. Rept. 44: 679-680.
18. Letterell, F. M., E. C. Tullis, R. T. Otten, and A. Gubernik. 1954. Physiologic races of *Piricularia oryzae* Cav. Phytopathology 44: 495. (Abstr.)
19. Leaver, F. W., J. Leal, and C. R. Brewer. 1947. Nutritional studies on *Piricularia oryzae*. J. Bacteriol. 54(4): 401-408.
20. Lee, Y. S. and L. C. Wu. 1967. The nitrogen nutrition of *Piricularia oryzae* Cav. Bot. Bull. Acad. Sinica 8(1): 66-72.
21. Otani, Y 1952 Growth factors and nitrogen sources of *Piricularia oryzae* Cav. Ann

Phytopath. Soc. Japan, 17(1): 9–15.

22. Otani, Y. 1953. Carbon sources of *Piricularia oryzae* Cav. Ann. Phytopath. Soc. Japan 17: 119–120.
23. Otsuka, H., K. Tamari, and N. Ogasawara. 1965. Variability of *Piricularia oryzae* in culture. p. 69–109. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
24. Suzuki H. 1967. Studies on biologic specialization in *Piricularia oryzae* Cav. Tokyo University of Agriculture and Technology, Tokyo. Japan. 235 p.
25. Tanaka, S. 1965. Nutrition of *Piricularia oryzae* in vitro. p. 23–33. In The Rice Blast Disease. Proc. Symp. at the Int. Rice Research Inst., July 1963. Johns Hopkins Press, Baltimore.
26. Tanaka, S., and H. Katsuki. 1951. Growth factors of the fungus causing rice blast disease. 1. Biotin as the principal growth factor. J. Chem. Soc. (Pure Chem. Sect.), Japan, 72(2/3): 231–233.
27. Tanaka, S., H. Katsuki, and F. Katsuki. 1952. Biochemical studies of the blast disease of rice plants IV. Effect of the different nitrogen sources in the culture media upon the nutritional absorption of the blast disease fungus. Ann. Phytopath. Soc. Japan, 16 (3/4): 103–106.
28. Tochinai, Y. and M. Shimamura. 1932. Studies on the physiological specialization in *Piricularia oryzae* Br et Cav. Ann Phytopath. Soc. Japan, 2: 414–441.
29. Tomizawa, C. and H. Koike. 1953. Studies on the nutritional physiology of the rice blast fungus. Ann. Phytopath. Soc. Japan, 17: 113–118.
30. Tseng, T. C. and L. C. Wu. 1967. The carbon nutrition of *Piricularia oryzae* Cav. Bot. Bull. Acad. Sinica 8(1):73–79.