PHYSIOLOGY OF PARASITISM

4. Rhizoctonia metabolites—Amino acids and others*

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Introduction

In the studies of of host-parasite interaction with mung bean-Rhizoctonia model, it was believed that type of infection or infection mechanism might be determined by the nature of metabolites produced by the pathogen (Wu, 1967, 1968). A regulatory effect of metabolites was evidenced by the fact that the mung bean grown on water agar containing culture filtrate of *Rhizoctonia solani* revealed similar patterns in their alterations of reducing sugar, amino acid, nucleic acid, and protein contents to those infected with *R. solani* per se (Wu, 1967).

The correlation among the growth, pathogenicity, and toxin production of *R. solani* was also demonstrated (Wu, 1965). The toxic substances synthesized by the fungus were known to reduce seed germination (Boosalis, 1947, 1950; Nishimura and Sasaki, 1963; Sherwood and Lindberg, 1962), inhibit development of secondary root (Boosalis, 1947, 1950; Sherwood and Lindberg, 1962; Lai and Wu, 1963), and eventually induce stunting of the host plants (Newton and Mayers, 1935; Lai and Wu, 1963; Wu, 1965). The extract obtained from the infected plants also displayed significant inhibitory effect on the growth of mung bean seedling as compared with that obtained from healthy plants (Lai and Wu, 1963; Wu, 1965). Apparently, these pathological alterations in Rhizoctonia-infected plants were related to the metabolites produced by the fungus. However, little is known so far about the Rhizoctonia metabolites products in Czapek's sucrose nitrate solution which we have used.

The purpose of the present investigation is to separate and identify the compounds synthesized by *R. solani* in this particular synthetic medium and determine whether these compounds possess growth regulating properties.

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Materials and Methods

An isolate of *Rhizoctonia solani* (ReCS5) highly pathogenic to mung bean seedling was used in the present investigation. Culture filtrate of the fungus was prepared by transferring three mycelial discs from the margin of colony, two-day old culture, of the fungus grown on potato-dextrose agar (PDA) to 500 ml Erlenmeyer flask containing 150 ml of Czapek's sucrose nitrate solution (Wu, 1965). These cultures were incubated in a room of 25–27°C for a given period of time. The culture filtrates were obtained by straining through two layers of cheese cloth before extraction and fractionation. Some batches of culture fluids were concentrated in vacuo below 45°C to remove water until the volume became 1/20 of the original volume before extraction. The mycelium from the above cultures was pressed to remove all residual fluid and stored in a freezer at −15°C until use.

Studies on the changes in hydrogen ion concentration, carbohydrate, amino acid, and toxicity of culture filtrates were carried out with 125 ml Erlenmeyer flask containing 50 ml of Czapek's sucrose nitrate solution, pH 7.6. Two-day old culture of *R. solani* grown on PDA at 25–27°C was served as the source of inocula. These cultures were incubated at 25–27°C for a given period of time. Ten flasks were harvested each time at intervals of four days. The culture filtrates were collected separately by pouring culture fluids in funnel with a piece of filter paper (Toyo filter paper No. 5B). An aliquot of culture filtrate was taken and analysed for the changes in hydrogen ion concentration with a Beckman Zeromatic II pH meter, total carbohydrate by colorimetric determination with anthrone reagent (Neish, 1952), amino acid by colorimetric method of Rosen (1957) with ninhydrin, and toxicity with mung bean. The mycelium was washed with 100 ml distilled water to remove residual medium and dried at 110°C for 20 hours and weighed.

For the analysis of amino acid, a Technicon automatic analyzer was used for automatic recording chromatogrpaphy and the method of Spackman *et al.* (1958) was adopted. For this purpose, one ml concentrated extract was diluted to two ml, added ten ml one per cent picric acid and centrifuged to clarify the extract. The supernatant was passed through Dowex 2–×8 column, washed with 0.02 N HCl for three times, five ml each time, and all efluents were collected. The efluent was concentrated to one ml in vacuo and then adjusted pH value to 2.2 with 1N NaOH as already described (Wu, 1969).

The stock solutions (10 mM) of 15 amino acids tested for their effect on the growth of mung bean were prepared by dissolving an adequate amount of each amino acid (Chromatography grade) either in distilled water or in 0.1N HCl as suggested by the manufacturer, Ajinomoto Co., and kept in a freezer at −15°C. They were glycine, DL-alanine, DL-valine, L-leucine, L-isoleucine,

L-serine, DL-threonine, L-phenylalanine, L-histidine, L-aspartic acid, L-glutamic acid, L-lysine, arginine, L-cystine, and DL-methionine which were detected in the culture filtrate of the fungus by an automatic amino acid analyzer in the present experiment.

The culture filterates were fractionated in the following manner: One liter of culture filtrate was acidified to pH 3.8 with 1N HCl (Sequeira, 1967) and washed in a separatory funnel with either 400 ml of peroxide free ethyl ether or ethyl acetate for four times. The water layer was designated as fraction 1. The organic layer was extracted with 4×400 ml of 5 per cent sodium bicarbonate (W/V), approximately pH 9.0, to separate extract into acidic and nonacidic components as suggested by Bentley (1962). The organic layer retaining nonacidic auxins together with the pigments was designated as fraction 2. The acidic compounds, such as best known auxins were recovered from the bicarbonate layer by acidifying to pH 3.4 with 5N HCl and re-extracting with peroxide free ethyl ether, 400 ml for four times since IAA was most efficiently recovered from an aqueous solution over a pH range 2.5 to 5.0 (Bentley, 1962). The alkaline water layer was designated as fraction 3 and the etheral layer as fraction 4. The peroxide of ethyl ether was detected and removed by ferrous sulphate as already described (Yu *et al.*, 1967). Extraction of mycelium was accomplished by blending the frozen samples with a volume of 70 per cent methanol ten times the wet weight of the mycelial mats and the extracts were evaporated in a rotary evaporator to remove methanol. Then the extract was fractionated as already mentioned in the case of culture filtrates.

Separation of each fraction from different sources was made by either paper chromatography on Whatman No. 1 filter paper or thin layer chromatography on Wakogel B-5 (Silica gel with 5% binder), 250 μ thick. For paper chromatography, solvent mixtures were mainly isopropanol, ammonia (28%), and water (8:1:1), occasionally n-butanol, acetic acid, and water (4:1:5) (Bentley, 1962) since the former was found to be much more satisfactory with the present samples. For thin layer chromatography, solvent systems were confined to alkaline system (methyl acetate–isopropanol–28% ammonia, 45:35:20) and acidic system (chloroform–99% acetic acid, 95:5) as suggested by Stahl (1963). Chromatograms were allowed to develop approximately 30 cm from the point of origin for paper chromatography and 10 cm for thin layer chromatography at 25–27°C. Chromatograms were illuminated with an U. V. lamp (2537Å) of Mineralight Model R-51 manufactured by Ultra-violet Prod. Inc. The fluorescent spots were marked and sprayed with Ehrlich's reagent (Bentley, 1962; Waldi, 1963), or occasionally, with some other spraying reagents listed by Waldi (1963) while unsprayed chromatograms were bioassayed.

Bioassay was carried out with mung bean, Avena coleoptile and leaf. Mung bean seeds were soaked in distilled water for one hour, and in an aqueous solution of 0.5 per cent mercuric chloride for another one hour, and then rinsed in several changes of sterile distilled water. The chromatogram was cut into pieces. Twenty seeds were placed in a 5 cm petri-dish, each with a piece of cut chromatogram unsprayed. Three or five ml distilled water were added to elute the compound tested, and incubated at 25–27°C for 40 to 48 hours before measurement. Avena coleoptile straight growth test and Avena leaf test were carried out as described by Bentley (1962). Either unhusked and husked oat seeds were surface-sterilized with 0.1 per cent mercucric chloride for two minutes, rinsed in several changes of sterile water. The treated seeds were placed in a petri-dish (12 cm in diameter and 10 cm high) with a piece of filter paper and 15 ml of distilled water. They were incubated in a box with a red fluorescent lamp (10 W) and allowed to germinate and grow for five days, approximately 30–50 mm high. The coleoptiles of equal length were cut into 6 mm sections at the level 6 mm below tip for the Avena coleoptile straight growth test and for Avena leaf test, 6 mm section was cut from the base of each coleoptile to include the node. Immediately after cutting, the sections were floated in sterile water and allowed to stand approximately one to two hours in the dark before use. The sections were distributed in 5 cm petri dishes, five to ten sections for each, with a piece of chromatogram and two ml sterile water. They were incubated at 25–27°C for 40 to 48 hours before measurement.

Absorption spectra in the region between 220 mμ to 400 mμ were determined on a Unicam SP 500 spectrophotometer at 2 mμ intervals. One portion of a chromatogram unsprayed was eluted with two or three ml 95 per cent ethanol or methanol depending on the size of paper strips.

Results

Hydrogen concentration, carbohydrate, and toxicity.—Changes in hydrogen concentration and carbohydrate during the growth of *R. solani* were not very striking (Table 1). The pH values of culture filtrates tended to decline at the beginning of incubation. However, this trend was no longer retained. The fluctuation was random with no consistent pattern in the later stage of cultivation. In this series of experiments, it was noted that the pH values of the culture fluids were often raised when the fluids became darker. Therefore, the shift in pH values might be attributed to the pigment formation in the culture filtrate.

The trend in total carbohydrate with age of the culture was more or less similar to that observed in the case of hydrogen ion concentration. Nothing

however, was known about the cause in the former case. The similarity was observed in the determination of toxicity with age of cultures (Table 1).

Table 1. *Effect of age on hydrogen ion concentration, carbohydrate, and toxicity in culture filtrate of* ***Rhizoctonia solani*** *grown on Czapek's sucrose nitrate solution at 25°C*

Age of culture (day)	pH value	Carbohydrate (gm/flask)	Growth (cm) of mung bean in		A/B ×100
			Culture filtrate (A)	Czapek's medium (B)	
4	7.6	1.35	2.5	2.7	92.7
8	7.3	1.23	2.3	2.7	85.2
12	7.1	1.17	2.5	2.6	96.0
16	6.1	1.33	2.5	2.7	92.7
20	5.5	1.31	1.8	2.6	69.3
24	5.8	1.57	2.1	2.6	80.6
28	4.9	1.31	2.0	2.6	76.8
32	6.1	1.24	1.7	2.6	65.4
36	5.5	1.20	1.2	2.5	48.0
40	5.8	0.80	1.4	2.6	53.8

Phytotoxic activity of the filtrate was detected as early as in four days old stationary cultures. However, it was not predominant until 20 days after inoculation. Thereafter, the toxicity was further increased though there were some fluctuated data of the toxicity with the age of cultures.

Amino acid analysis—Changes in amino acid content in culture filtrates were very striking 16 days after inoculation. In the initial stage, amino acid accounted for very low level. The similar pattern was also observed in the growth of mycelium. The trend was better visualized when the data were plotted on a graph (Fig. 1). Within the 12-day incubation, there was a tendency towards an increase in the rate of dry mycelium whereas the amino acid accumulation in culture filtrate was little. But by far the most striking and significant of the dry weight changes in mycelium brought about an increase in the accumulation of amino acids.

Fifteen amino acids were detected in the present experiment (Table 2). They were glutamic acid, alanine, glycine, valine, threonine, cystine, aspartic acid, serine, isoleucine, leucine, arginine, histidine, lysine, methionine, and phenylalanine. Among 15 amino acids detected, glutamic acid, alanine, glycine, valine, threonine, cystine, and aspartic acid were found to be over 10 n moles per ml of concentrated filtrates of three weeks old culture. As shown in Table 3, the relative amount of amino acid in the filtrate of two weeks old culture was different from that of three weeks old culture. In two-week old culture,

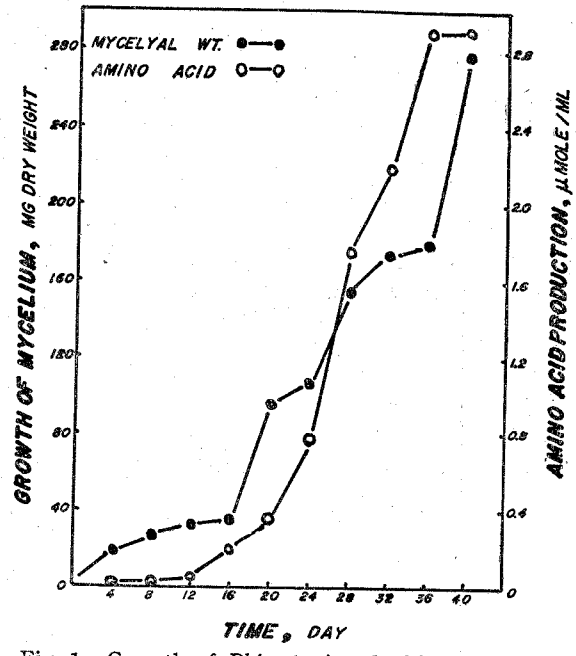

Fig. 1. Growth of *Rhizoctonia solani* in relation to amino acid production

Table 2. *Amino acids produced in culture filtrate of* ***Rhizoctonia solani*** *grown on Czapek's sucrose nitrate solution at 25°C for three weeks.**

Amino acid	Concentration (n mole/ml concentrate)
Glutamic acid	63.5
Alanine	58.7
Glycine	27.9
Valine	22.9
Threonine	19.7
Cystine	19.7
Aspartic acid	12.8
Serine	9.4
Isoleucine	8.8
Leucine	6.4
Arginine	4.8
Histidine	4.5
Lysine	3.6
Methionine	2.2
Phenylalanine	1.8

* The culture filtrate was concentrated with a rotary evaporator in vacuo below 45°C to remove water until the volume became 1/20 of the original volume.

Table 3. *Comparative amino acid compositions in culture filtrate of* ***Rhizoctonia solani*** *grown on Czapek's sucrose nitrate solution at 25°C for two and three weeks**

Amino acid	Relative amount of amino acid (%)**	
	Two-week	Three-week
Alanine	30.75	22.00
Glutamic acid	23.20	23.68
Glycine	7.91	10.45
Leucine	6.20	2.40
Valine	4.95	8.57
Threonine	4.86	7.37
Serine	4.16	3.52
Isoleucine	4.07	3.30
Aspartic acid	3.08	4.80
Lysine	3.07	1.35
Phenylalanine	2.39	0.67
Cystine	2.32	7.37
Arginine	1.45	1.80
Histidine	1.18	1.70
Methionine	0.49	0.82

* Toxicity (per cent growth of mung bean over control): 66.9% for 2-week and 28.8% for 3-week old culture filtrates.

** These values are per cent of total n moles of the listed amino acids.

alanine accounted for 30.75 per cent of total amino acid whereas 22.00 per cent was found in three-week old culture. Glutamic acid kept same level, i. e. from 23.20 per cent to 23.68 per cent. It is worthy to mention that the concentrations of valine, threonine, methionine, and cystine in two-week old culture were about half as much as those in the three-week old culture. On the other hand, the relative amounts of leucine, lysine, and phenylalanine in three-week old cultures were decreased to narly one third those of two-week old cultures.

Amino acid as a plant growth regulator.—Attempts to determine the regulatory effect of amino acids on the growth of mung bean were complicated by random fluctuations which occurred among replicates at different concentrations of amino acid mixture (Table 4). A mixture of 15 amino acids, detected in the present experiment (Table 2 and 3), were prepared by mixing same volume of 10 mM solution of each amino acid. Dilutions were made with sterile water to secure a given concentration, i. e. 0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, and 10.0 mM solutions, which were within the range of concentrations found in the filtrate of *R. solani*.

As shown in Table 5, the growth of mung bean varied with different

amino acids under different conditions at the concentration of one mM. DL-threonine stimulated the growth of mung bean 112 and 117 per cent over the control plant respectively in the light and the dark. DL-valine and L-isoleucine were stimulative in the dark and glycine in the light. The inhibitory effect

Table 4. *Effect of amino acid on the growth of mung bean incubated at 25°C for two days**

Concentration of amino acid (mM)	Growth of mung bean (cm)
0	3.95
0.2	3.89
0.4	2.88
0.6	2.91
0.8	2.85
1.0	2.94
2.0	3.02
4.0	2.85
10.0	1.77

* A mixture of 15 amino acids found in the culture filtrate of ***Rhizoctonia solani*** was made at equal rate before assay.

Table 5. *Effect of different amino acids on the growth of mung bean incubated at 22°C for two days in the light and dark*

Amino acid*	Growth (cm) of mung bean in	
	Light	Dark
Glycine	2.06	2.32
DL-Alanine	1.43	1.82
DL-Valine	1.79	2.78
L-Leucine	1.43	2.28
L-Isoleucine	1.86	2.49
L-Serine	1.67	1.69
DL-Threonine	2.04	2.53
L-Phenylalanine	1.48	2.42
L-Histidine	1.68	2.42
L-Aspartic acid	1.61	2.60
L-Glutamic acid	1.41	2.73
L-Lysine	1.63	2.57
Arginine	1.49	2.41
L-Cystine	1.13	0.91
DL-Methionine	1.63	1.71
Control	1.82	2.36

* The concentration was one mM.

of DL-alanine, L-serine, L-cystine, and DL-methionine was displayed in the light and the dark whereas L-leucine, L-phenylalanine, L-histidine and arginine were effective only in the light. L-Aspartic acid, L-glutamic, and L-lysine behaved differently with the testing environment. In the light, they inhibited the growth of mung bean but they became stimulative when they were tested in the dark.

In this connection, changes in the toxicity of mixed filtrates obtained from different batches of cultures are worthy to mention (Table 6). It is possible that the different batches of culture filtrates differed in their relative amounts of amino acids since the comparative amino acid compositions in the filtrates of two and three weeks old cultures were varied. Different amino acids might behave differently at different concentrations. This might explain the disappearance of the phytotoxic activity when the filtrates of different batches of cultures were mixed and assayed.

Table 6. *Effect of culture filtrates from different batches of* ***Rhizoctonia solani*** *on the growth of mung bean.*

Sample*	Growth (cm) of mung bean	Inhibition (%)
1	1.39	41.60
2	2.16	9.25
3	1.81	23.95
1+2+3	2.30	3.36
Control	2.38	0

* All the concentrated sample filtrates were diluted to 1/20 with sterile water before assay.

Regulators other than amino acids.—Twenty liters of culture filtrates were evaporated in vacuo below 45°C in a rotary evaporater to one liter, 1/20 of original volume. The concentrated filtrate was fractionated into four fractions as aforementioned. Each fraction was evaporated in vacuo below 45°C to less than five ml and stored in a freezer of -15°C until use. The concentrated extracts were applied to Whatman No. 1 filter paper, ten microliters for each spot. Fifty micrograms of synthetic indoleacetic acid (IAA) and gibberellic acid (GA) were co-chromatographed for each run.

After development and drying of the paper, the positions of the individual bands were firstly located by their fluorescence in the ultraviolet (U. V.) before and after exposed to ammonia vapor. Then they were located by spraying the chromatograms with Ehrlich's reagent after a ninhydrin reaction was carried out. The developed chromatogram was also cut into six strips and each strip was divided into 15 sections. Each section was placed in a 5 cm

Petri-dish with three ml sterile water and 20 grains of mung bean seeds. The resultant data (Table 7) indicated that auxin like substance was located at Rf 0.63 which was identical with that of authentic IAA though it was Ehrlich negative reaction, with fluorescence under a U. V. lamp, which might be due to the low concentration of auxin in the eluate. At Rf 0.77, there was gibberellin-like substance in fractions 2, and 4, however, fluorescence in U. V. light was not developed after treating with H_2SO_4 which was sensitive to five micrograms GA (Bentley, 1962).

Table 7. *Effect of chromatographed extracts obtained from culture filtrate of* ***Rhizoctonia solani*** *on the growth of mung bean**

Rf-avlue	Growth (cm) of mung bean in					
	Original filtrate	Fraction 1	Fraction 2	Fraction 4	IAA	GA
0.03	2.04	2.31	2.64	2.81	2.02	2.64
0.10	2.76	2.96	1.99	2.07	—	2.87
0.17	2.71	2.46	2.34	2.95	2.75	2.79
0.23	2.99	2.57	2.51	3.51	2.52	3.31
0.30	2.79	2.04	2.48	3.23	2.15	2.31
0.37	2.52	1.62	2.16	2.50	1.98	2.54
0.43	2.41	2.11	2.38	2.12	2.73	2.76
0.50	2.28	2.19	2.26	3.12	1.94	2.33
0.57	2.28	1.88	3.10	2.68	1.65	2.07
0.63	2.83	2.83	3.16	2.39	2.14	2.72
0.70	2.95	2.12	2.14	1.49	2.91	2.97
0.77	2.12	2.13	2.82	2.67	2.57	3.06
0.83	2.53	2.56	2.51	2.12	2.92	2.23
0.90	2.56	2.53	2.60	2.39	2.12	2.64
0.97	2.34	2.25	—	2.37	2.21	2.53

* The growth of control bean was 2.54 cm. One liter of concentrated culture filtrate was acidified to pH 3.8 with IN HCI and washed in a separatory funnel with 400 ml of peroxide free ethyl ether for four times. The water layer was designated as fraction 1. The organic layer was extracted with 4×400 ml of 5 per cent sodium bicarbonate (W/V) and the organic layer was designated as fraction 2. The alkaline solution was acidified to pH 3.4 with 5N HCI and extracted with peroxide free ethyl ether, 400 ml for four times. The alkaline water layer was designated as fraction 3 and the etheral layer as fraction 4.

It was speculated that storage and evaporation of culture filtrates and composition of medium might result in the failure of identification of fungal metabolites. Thus, three samples (Table 8) were fractionated without preliminary concentration of the culture filtrates. Sample #2 was extracted one day after harvest and sample #3 and #4 were stored prior to extraction in a

cold room about 2-5°C for eight days after harvest. In sample #4, potato decoction, 200 grams per liter, was added to Czapek's sucrose nitrate solution to compare with those without potato decoction, i. e. sample #2 and 3. Each sample consisted of culture filtrate and mycelial mats. To detect acidic compunds, such as IAA and GA, only fraction 4 was tested for each sample.

Table 8. *Effect of chromatographed extracts obtained from culture filtrates and mycelial mats of* ***Rhizoctonia solani*** *on the growth of mung bean**

Rf-value	Growth (cm) of mung bean							
	Culture filtrate			Mycelial mats			Authentic	
	#2	#3	#4	M#2	M#3	M#4	IAA	GA
0.03	2.01	3.52	2.30	2.80	2.25	2.87	3.15	2.70
0.08	4.48	2.94	3.46	1.95	3.78	2.53	3.22	2.92
0.13	2.81	4.10	2.94	1.75	2.61	4.09	4.28	2.94
0.18	2.84	3.13	2.84	4.25	3.56	3.22	3.26	4.52
0.23	2.50	2.34	2.50	3.53	3.37	2.46	3.80	2.98
0.28	3.01	3.30	3.03	2.87	2.82	1.69	3.20	3.68
0.33	3.76	2.64	2.47	3.30	2.16	2.59	4.00	3.35
0.38	4.34	3.61	2.29	4.33	3.39	3.17	3.04	2.41
0.43	2.24	3.72	1.43	2.94	1.92	3.59	3.53	3.86
0.48	3.62	2.61	2.76	3.25	1.52	3.74	2.74	3.15
0.53	3.52	2.08	3.15	2.80	2.65	3.71	1.08	2.29
0.58	3.15	3.42	3.60	2.45	3.33	3.03	1.66	2.82
0.63	3.29	3.32	1.74	2.40	2.82	1.23	2.86	3.65
0.68	5.15	2.48	3.23	3.09	2.19	3.88	3.24	2.46
0.73	5.04	2.69	3.52	3.52	3.15	3.32	3.06	4.51
0.78	2.08	1.27	3.13	1.65	2.69	2.58	3.92	3.24
0.83	2.85	2.82	3.08	3.30	3.01	2.18	3.43	3.13
0.88	4.44	2.95	4.28	—	3.45	3.15	2.75	2.71
0.93	3.50	3.44	4.29	4.46	3.70	1.66	1.52	3.74
0.98	2.88	3.43	4.64	4.30	1.48	3.37	2.85	2.87

* The growth of control bean was 2.45 cm. Sample #4 contained potato decoction, 200 gm per liter. Only fraction 4 was tested. The mycelial mats were blended with a volume of 70 per cent methanol ten times the wet weight of the mycelial mats and the extracts were evaporated in a rotary evaporator to remove methanol. Then the extract was fractionated as already described in the case of culture filtrate.

As shown in Table 8, extractions derived from mycelial mats contained more compounds than those from culture filtrates. Neither IAA nor GA-like substances were found. Therefore, only those bands located by their fluorescence in ultraviolet were assayed with mung bean, Avena coleoptile section, Avena leaf and measured for their U. V. spectra. The results showed that

there was substance with Rf value 0.97 stimulative to the growth of mung bean, Avena coleoptile section, and Avena leaf, yet the effects on Avena coleoptile and leaf were not consistent (Table 9). Study of U. V. spectra indicated that the absorption maximun was nearly at 250 mμ. This substance was detected in the fraction 4 of all samples tested, which might be destroyed during the course of evaporation. Further study on the culture filtrate of sample #4, which contained potato decoction, revealed that inclusion of the medium caused the complication of the fungal metabolites.

Table 9. *Effect of fluorescent substances obtained from culture filtrates and mycelial mats of* ***Rhizoctonia solani*** *on the growth of mung bean**

Rf-value	Growth (cm) of mung bean						
	Culture filtrate			Mycelial mats			Authentic
	#2	#3	#4	M#2	M#3	M#4	IAA
0.00	2.70	2.40	2.62	2.40	1.54	1.78	2.32
0.08	1.35	2.98	2.77	3.02	2.61	2.08	—
0.10	—	—	—	—	—	2.06	—
0.16	—	—	—	—	—	0.76	—
0.22	—	—	—	—	—	2.00	—
0.23	—	—	—	2.20	2.61	—	—
0.42	—	2.85	2.66	—	—	—	—
0.47	—	—	—	—	—	—	0.81
0.97	2.81	3.01	3.31	3.18	2.39	2.32	2.11

* The growth of control bean was 2.24 cm.

In the foregoing experiments, only three weeks old cultures were examined since the phytotoxic activity of the culture filtrate was found to be predominant 20 days after inoculation. Younger cultures of *R. solani*, grown for one and two weeks, were also analysed to secure the production of auxin-like substance in the culture. The results obtained from Avena coleoptile straight growth test showed that there was auxin-like substance with Rf value from 0.57 to 0.70 which was identical with that of synthetic IAA co-chromatographed.

Because of the shorter chromatography time and high detectability of the substances, thin layer chromatography was used to direct the paper chromatography in progress. The results indicated that there were steroids, terpenes, flavonoids, and indole derivatives in both culture filtrate and mycelium of *R. solani*. Identification of these compounds are under way.

Discussion

In the previous report (Wu, 1968, 1969), it was found that amino acid pool was considerably affected in the early stage of disease development. Thus the

changes in amino acid content of the culture filtrates of *Rhizoctonia solani* were studied with age of the culture. Subsequently the amino acid composition of culture filtrate was studied by an automatic amino acid analyzer. Fifteen amino acids were identified in the concentrated filtrates of the fungus. They were glutamic acid, alanine, glycine, valine, threonine, cystine, aspartic acid, serine, isoleucine, leucine, arginine, histidine, lysine, methionine, and phenylalanine, Among fifteen amino acids, threonine, serine, and cystine were not found in both healthy and infected mung bean, whereas proline and tyrosine were not detected though they were present in the mung bean (Wu, 1969).

Amino acids in relation to plant diseases were thoroughly discussed by van Andel (1966). However, there was little light on the role of amino acids in establishment of Rhizoctonia infection. In the present experiment, it was shown that the growth of mung bean was strikingly affected by synthetic amino acids which were found in the culture filtrate of *R. solani*. Nevertheless the regulatory effect of the amino acid varied with different amino acids under different conditions, e. g. in the light and the dark. Changes in the toxicity of the mixed filtrates obtained from different batches of cultures were also found since the composition of amino acids in two weeks old cultures was different from that in three weeks old cultures. The effect might be applicable to explain why different dilutions of culture filtrate displayed different effects on the plants in their chemical patterns (Wu, 1967).

Effect of chromatographed extracts obtained from both culture filtrate and mycelium of *R. solani* showed that there were a complex of plant growth regulators which might induce or facilitate the Rhizoctonia infection. Although there were many genera and species of fungi being examined for auxin production (Gruen, 1959), little was known about their exact role played in plant infection (Sequeira, 1963). In the present study, auxin- and gibberellin-like substances were detected in culture filtrate of *R. solani*. Nevertheless, a substance with the absorption maximum at 250 mμ was found to be stimulative to the growth of mung bean. Since synthetic substance was not available during this study, identification of this substance must remain tentative.

Summary

Changes in hydrogen concentrations and carbohydrates were not very striking during the growth of *Rhizoctonia solani*. They tended to decline with age of the culture. Phytotoxic activity of the filtrate appeared as early as in four days old culture. However, it was not predominant until 20 days after inoculation. In the initial stage amino acid level was very low while the accumulation of amino acids was brought about by significant increase in the dry weight of mycelium, i. e. 12 days after inoculation.

Fifteen amino acids were detected in the present experiment. They were glutamic acid, alanine, glycine, valine, threonine, cystine, aspartic acid, serine, isoleucine, leucine, arginine, histidine, lysine, methionine, and phenylalanine. Among the 15 amino acids, the concentrations of alanine and glutamic acid were the highest. Amino acids seemed to regulate the growth of mung bean in vitro. DL-threonine stimulated the growth whereas DL-alanine, L-serine, L-cystine, and DL-methionine displayed the inhibitory effect on the growth of mung bean. The effect of amino acids varied with different assay conditions.

Auxin- and gibberellin-like substances were identified in the culture filtrates of *R. solani*. Chromatographic and spectrophotometric analyses together with bioassay showed the presence of some compounds of different nature in culture filtrates and mycelium of *R. solani*.

寄 生 生 理

4. 苗立枯病菌代謝物—氨基酸及他物

吳 龍 溪

苗立枯病菌在生長過程中，其培養液酸鹼度，碳水化合物含量之變化不大。但在初期具有遞減之趨勢，至色素之產生後，即發生不規則之變化，培養液之毒性在培養四日即可測知，至二十日後才顯著加强。氨基酸含量在培養初期不多，至十二日後菌絲乾重量突然增加而顯著增高。

經分析結果獲知在苗立枯病菌培養液含有十五種氨基酸：麩氨酸、氨基丙酸、氨基乙酸、甲型氨基異戊酸、滋利氨酸、胱氨酸、天門冬酸、絲氨酸、異白氨基酸、白氨基酸、鮭卵酸、纈氨酸、離氨基酸、甲硫氨酸以及苯氨基丙酸，其中以氨基丙酸及麩氨酸之含量爲最高。

以十五種氨基酸水溶液測定綠豆發芽生長時，發現氨基丙酸、絲氨酸、胱氨酸以及甲硫氨酸，具有抑制作用。但滋利氨酸却有促進作用。一般而言，氨基酸對綠豆生長具有抑制作用，但易受環境支配而改變其生理作用，值得注意。在本菌菌絲及其培養液中所含有生長素及抑制物質之性質，尙須詳細研究確定。

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