FACTORS AFFECTING THE ACTIVITY OF PECTIC ENZYMES PRODUCED BY *THANATEPHORUS CUCUMERIS* (FRANK) DONK FROM SOYBEAN PLANT*

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Introduction

Thanatephorus cucumeris (Frank) Donk is omnivorous and subsists saprophytically in the soil. Damping-off, stem canker, root rot, storage-organ decay, and foliage blight or spot caused by this fungus were reported elsewhere (Chen *et al.* 1963, Flenje and Hagedorn 1964, Ryker and Gooch 1938, Gonzalez and Dwen 1963, Lai and Wu 1663, Boosalis 1947, 1950, Nakayama 1940, Chen 1963). Han (1966), and Wu and Lin (1967) reported that the fungus attacked soybean plant causing a serious aerial blight and root rot in Taiwan. Several reports were published concerning the growth, pathogenicity, and toxin production of *Rhizoctonia solani* in cultures (Sherwood and Lindberg 1962, Wyllie 1962, Lai and Wu 1953). Fungal metabolism with the production of oxidases, cellulolytic and pectic enzymes by *R. solani* have been studied by many investigators (Garrett 1962, Barker and Walker 1962, Tweedy and Poweedy 1962, Mann 1962, Bateman, 1962, 1963, 1964, Bateman and Maxwell 1965, Maxwell and Bateman 1967, van Etten *et al.* 1967, Boosalis 1947, 1950, Woodbury *et al.* 1962). The majority of investigators demonstrated that the pathogenicity of *T. cucumeris* was in part due to the action of pectic enzyme produced by the fungus (Barker and Walker 1962, 1963, van Etten *et al.* 1967, Newton and Mayers 1935).

The present investigation was to compare the pathogenicity among isolates, to study the influence of the culture conditions on the ability to produce pectic enzymes by the test fungus and to study the nutritional factors affecting the enzyme activities in liquid cultures.

Materials and Methods

Pathogenicity and pectic enzyme activity of the fungus: Soybean variety Chung-Hsing No. 2, the test plants used in this experiment, was grown in clay pots (5 in. dia.) in greenhouse. Inoculation of soybean leaves with different isolates was made for comparing their pathogenicity. The isolates of *T. cucumeris* used in this investiga-

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tion were isolated from soybeans showing typical symptoms of aerial blight collected from different localities in Taiwan. They were maintained on potato sucrose agar. In this experiment, agar disks of 0.5 cm in diameter supporting active growing mycelium cut from margin of the colonies were served as the inoculum. The inoculated plants were kept in a moist chamber. Pathogenicity of the isolates were read three days after inoculations, it was determined by the degree of severity on symptom expression. Pectic enzymes produced in infected tissue were assayed by the methods described by Bateman (1963). Each of 5 g leaf samples cut from lesions of infected leaves was homogenized in 10 ml of 0.5 N NaCl solution in a mortar. The homogenate was strained through 4 layers of cheesecloth to remove excess of debris, and the tissue extracts were centrifuged at room temperature for 20 minutes at 2000 rpm (3300 g) in a sorvall centrifuge. The supernatant, after dialyzed in ion-free distilled water for 25 hours at 5°C, was used for enzyme assay. Uninoculated leaves of the same age incubated at the same condition served as the control.

Factors affecting enzyme production in liquid cultures: Czapek's solution was used as basal medium throughout the experiment except otherwise stated. The mycelium, grown in Czapek's solution at 30°C for 2 days was rinsed in sterilized water and fragmented in water to make mycelium suspension, was used as the inoculum. Each culture was inoculated with 1 ml mycelium suspension. The medium was kept at pH 7.0, because this pH was found to be the optimum range for growth of the fungus (Han, 1966). The fungus was cultured in flasks containing 30 ml of liquid medium for 7 days at 30°C. At the end of 7th day, mycelium was removed from liquid cultures by filtration through Azume filter paper No. 1 with the aid of porcelian Buchner funnel and a partial vacuum. The filtrate was used for enzyme analysis to compare the pectic enzyme activity of different isolates. The temperature influence to enzyme production was determined by placing the cultures in incubators with different temperatures ranging from 4 to 44°C with 4°C intervals. The effect of pH on enzyme production was studied by culturing the fungus in media of different pH values adjusted by means of NaOH or HCl. In carbon source tests, several kinds of monosaccharides, oligosaccharides and polysaccharides were used. The culture media were autoclaved under 10 lb pressure for 40 minutes. Several kinds of inorganic and organic nitrogen compounds including amino acids were separately incorperated into media as nitrogen sources.

Enzyme assay: Pectin methyl esterase (PME) activity was determined by method modified from Smith (1958). The pH of the culture filtrates was adjusted to 7.0. To 1 ml of filtrate was mixed with 20 ml of 1% pectin solution containing 0.1 M NaCl and placed in room temperature (30°C) for 30 minutes. The pH of the reacted mixtures was immediately measured by pH meter. PME activity was determined by measuring the rate of pH reduction due to acid produced in the pectin substrate by the action of culture filtrate. Polygalacturonase (PG) activity was studied by the reduction in viscosity of the substrate on incubation with the enzyme preparation. The formula, $A=T_0-T_{20}/T_0-T_w\times 100$ (Fukumoto 1956), was employed. The thermal

inactivation of PG was made by incubating the enzyme preparations in test tube in water bath at 100°C for 10 minutes. The reaction mixture consisted of 20 ml of 0.5% pectic acid solution and 1 ml of enzyme solutions. Viscosity of the reaction mixtures was measured 20 minutes after mixing. End-group method as described by Kertesz (1955) was also employed for determining PG activity in some experiments. Under this method, 20 ml of 0.5% pectic acid previously adjusted to pH 4.0 at 26°C was mixed with 1 ml of enzyme solution and added to 0.2 ml of 1 M Na_2CO_3. 5 drops (each drop equal to 0.033 ml) of 0.1 N iodine solution was added, thoroughly mixed, and was incubated for 20 minutes. Finally 0.5 ml of 2 M H_2SO_4 was added and kept in room condition for 20 minutes. 5 ml of fresh starch solution (0.5 g in 100 ml water) was added in the mixture before titration. The excess of iodine was titrated with 0.1 N $Na_2S_2O_3$ solution. PG activity was expressed as PG. u./ml, indicating the millimoles of reducing groups liberated per minute per milliliter of enzyme solution.

Experiment Results

1. Pathogenicity and pectic enzyme activity of the isolates

In the experiments of pectic enzyme activities with healthy and infected tissue, the results are closely similar to that described by Bateman (1963). Higher content of PG was found in diseased tissue than in healthy tissue. PME was found in both samples, however, no significance was found to the pathogenicity of the causal fungus in soybean leaves. PG activities from lesion extracts were 5.551 and 4.478 relative units by isolates S 15-16 and S 30-1 respectively, while healthy tissue extracts contained from 0.596 to 2.21 relative units only (Table 1). Virulence of the isolates was compared by inoculating the soybean leaves with agar disk supporting active mycelium. The results indicated that the isolates of *T. cucumeris* possess different levels of pathogenicity in terms of leaf blight symptoms. Relationship between pathogenicity and the pectic enzyme activities was studied from culture filtrates of different isolates. Table 2 showed that high virulent isolates, in general, were capable of producing higher amount of PG in culture filtrates. PME showed significantly no correlation with the pathogenesis.

Table 1. Pectic enzyme activities in tissue of soybean leaf infected by *T. cucumeris* 3 days after inoculation

Isolates	Tissue sample[1]	PME[2]	PG[3]
Check	Healthy	0.92	0.596
S 15-16	Diseased	0.29	5.551
Check	Healthy	0.12	2.210
S 30-1	Diseased	0.30	4.478

1. Bateman's (1963) method of enzyme preparation was employed.
2. PME activity was determined by pH difference between pectin substrate by the action of filtrate and untreated one.
3. PG activity was determined from percentage reduction in viscosity.

Table 2 Pathogenicity of *T. cucumeris* on soybean leaves and pectic enzyme activity of different isolates cultured in Czapek's solution incubated for 7 days at 30°C.

Isolates	Relative pathogenicity [1]	pH of culture filtrate [2]	PME activity [3]	PG activity [4]
S 13-2 a	++	6.98	0.52	25.00
S 13-2 b	+++++	6.32	0.80	53.46
S 14-2	+++	7.10	1.22	25.96
S 14-4 a	++	6.90	0.63	31.67
S 15-4	+++++	6.93	0.42	74.82
S 15-6	+	6.80	0.49	5.28
S 15-7	+	6.65	0.88	6.27
S 15-8	+++	7.18	1.28	49.70
S 15-9	+++++	6.66	0.75	54.50
S 15-10	+++	6.93	1.15	49.59
S 15-11	++++	6.60	0.6	56.33
S 15-13	+++++	6.10	1.15	81.28
S 15-15	+++++	5.95	0.43	59.20
S 15-16	++++	6.86	1.35	45.71
S 15-17	++++	6.95	0.76	57.19

1. Inoculations were made on soybean leaves of eight plants in the same age. Agar disc supporting active mycelium served as the inoculum. "+~+++++" represents the degree of pathogenicity of the isolates.
2. Initial pH of the cultures were 7.0. All the pH of the cultures after incubation were measured and readjusted to 7.0 before enzyme assay.
3. 4. See Table 1.

2. Factor affecting pectic enzyme activities in culture

To understand the effects of culture age on the amount of enzyme production, filtrates were made from cultures incubated for different periods of time. The results indicated that the filtrates contained no detectable pectin methylesterase and polygalacturonase during the first 24-hours. Very little amount of enzymes in culture was yielded 2 days after incubation. A rapid increase of the enzymes were found in cultures incubated for 3 days. Highest amount of enzymes were detected from 7-day cultures (Fig. 1).

Enzyme production was relatively higher in temperature that was optimum for mycelial growth in liquid culture. 28~32°C was the optimum temperature favorable for PME production. 20~32°C is optimal for PG production, particularly at 28°C and 30°C. (Fig. 2, 3, 4).

pH 5.0 and 5.5 was most suitable for the production of PME and PG. No PME was detected from culture filtrates at the pH below 3.5 and above 8.0 (Fig. 5). The culture filtrates contained no detectable PG at the pH below 4.0 and above 8.5 at the experimental conditions, although mycelial growth was found under these conditions (Fig. 6). The influence of nutrients on enzyme production is summerized in Table 3

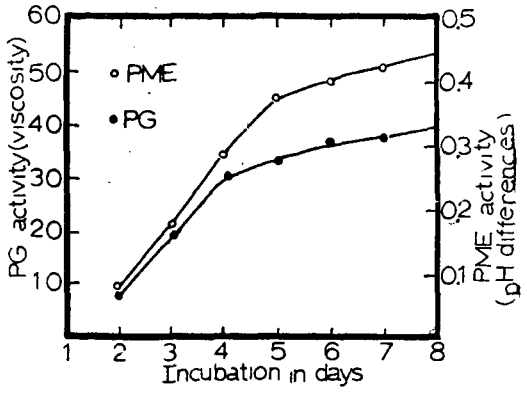

Fig. 1. Pectic enzymes detected from liquid culture incubated for different periods of time at 30°C.

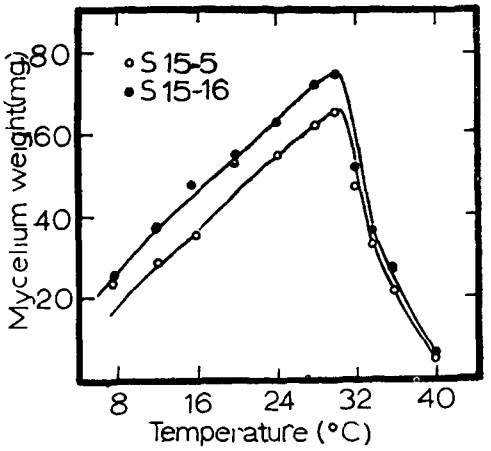

Fig. 2. Temperature in relation to mycelial growth in dry weight of two isolates (S 15-5, S 15-16) of *T. cucumeris* cultured in Czapek's solution incubated for 7 days.

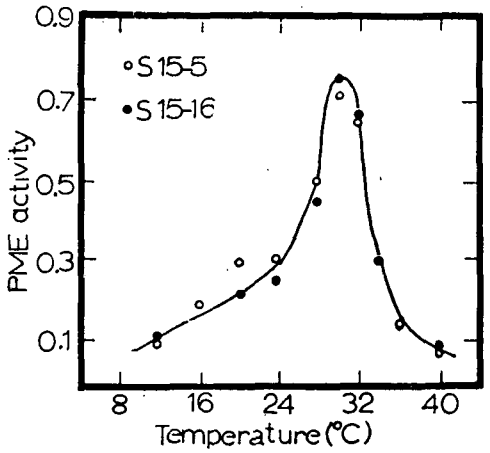

Fig. 3. Effect of temperature on PME activity of two isolates (S 15-5, S 15-16) of *T. cucumeris*. Enzyme activity was determined from pH differences between pectin substrate by the action of filtrate and untreated one.

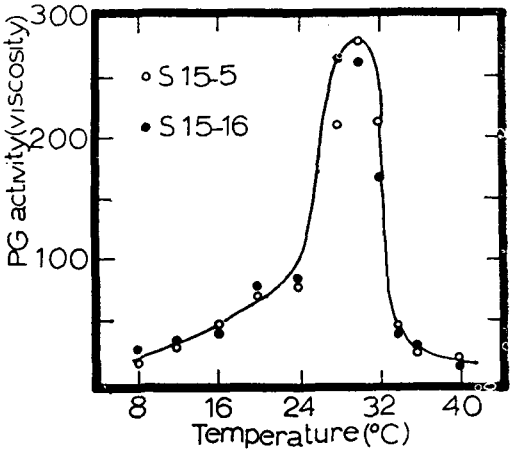

Fig. 4. Effect of temperature on PG activity of two isolates (S 15-5, S 15-16) of *T. cucumeris* in Czapek's solution 7 days after incubation. Enzyme activity was determined from percentage reduction in viscosity.

which shows that pectic acid, sorbitol, sorbose, xylose, dextrose, rhamnose and lactose are useful for the production of PME by the isolates tested. Arabinose, rhamnose, mannose, glycerol, soluble starch and galactose were found to be favorable for PG production. In the experiments with nitrogen sources, some inorganic compound, in general, were found to be more favorable for pectic enzyme production than organic compounds. Ammonium chloride and ammonium tartrate gave best results on PME production. So far as PG is concerned, ammonium phosphate, ammonium oxalate,

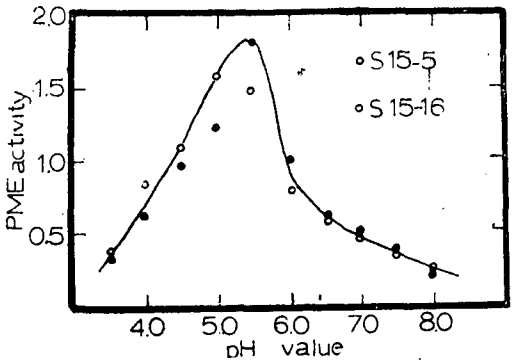

Fig. 5. The influence of pH values of culture medium on PME activity of two isolates of *T. cucumeris* in Czapek's solution incubated for 7 days at 30°C. The enzyme activity was determined from pH differences between pectin substrate by the action of filtrate and untreated one.

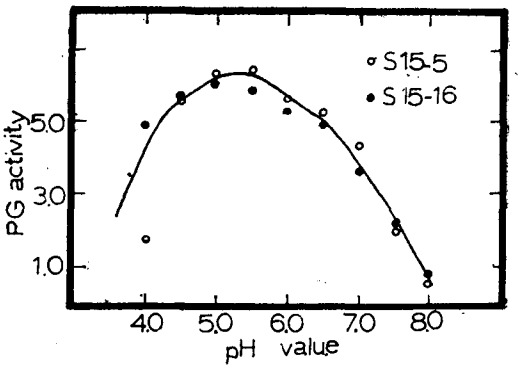

Fig. 6. The influence of pH value of culture medium on PG activity of two isolates of *T. cucumeris* in Czapek's solution incubated for 7 days at 30°C. The enzyme activity was determined from percentage reduction in viscosity.

Table 3. Effects of carbon sources on PME and PG activities of *T. cucumeris* in liquid cultures incubated for 7 days at 30°C.[1]

C-Sources.	Isolates S 15-5		Isolate S 15-16	
	PME[2]	PG[3]	PME	PG
Arabinose	0.50	69.39	0.30	48.41
Fructose	0.25	40.34	0.70	40.34
Galactose	0.60	44.38	0.25	44.38
Inositol	0.55	30.66	0.30	28.24
Mannose	0.55	44.38	0.60	48.41
Xylose	0.80	40.34	0.80	36.31
Dextrose	0.95	44.38	0.65	40.34
Lactose	0.60	37.12	0.85	36.31
Maltose	0.45	28.24	0.40	44.38
Saccharose	0.45	48.41	0.10	40.34
Dextrin	0.10	36.31	0	36.31
Inulin	0.30	28.24	0.50	32.28
Mannitol	0.60	44.38	0.50	24.21
Raffinose	0.40	48.41	0.45	40.34
Soluble Starch	0.60	64.55	0.50	40.34
Pectic acid	2.45	40.34	2.45	44.38
Dulcitol	0.35	32.27	0.25	36.31
Rhamnose	0.85	72.62	0.85	68.58
Salicin	0.55	37.92	0.50	36.31
Sorbose	0.95	40.34	1.05	40.34
Sorbitol	0.95	40.34	0.95	37.15

1. Figures in the Table are average of four replicates. Initial pH of the culture was 7.0 and the final pH of culture filtrates were measured 7 days after incubation. The final pH were readjusted to 7.0 before enzyme assay.

2. 3. See Table 1.

Table 4. Effects of some inorganic and organic nitrogen sources on relative activity of PME produced by *T. cucumeris* in liquid culture incubated for 7 days at 30°C.

Inorganic and organic nitrogen sources.	Isolates and enzymes			
	S 15-5		S 15-16	
	PME [1]	PG [2]	PME	PG
Sodium nitrate	0.17	100.0	0.08	96.4
Sodium nitrite	0.17	42.85	0.26	45.0
Calcium nitrate	0.68	22.0	0.71	28.0
Ammonium sulphate	1.83	50.0	1.45	47.5
Ammonium chloride	1.73	23.0	0.45	22.5
Ammonium phosphate	0.74	0	0.22	1.3
Ammonium nitrate	0.28	20.0	0.55	22.6
Potassium nitrate	0.83	100.0	0.06	100.0
Potassium nitrite	0.28	47.0	0.22	48.0
Ammonium tartrate	1.49	12.0	1.22	19.7
Ammonium oxalate	0.64	8.0	0.64	6.2
Ammonium acetate	0.04	0	0.27	0
Urea	0.35	22.85	0.35	25.1
Acetamide	0.43	38.57	0.47	40.2
Glycocoll	0.72	100.0	0.50	100.0

1. 2. See Table 1.

ammonium acetate, etc. seem to be of no use to PG production, whereas sodium nitrate, potassium nitrate and glycocoll gave the best results (Table 4). Among the amino acids tested, aspartic acid, glutamic acid, glutamine, isoleucine, leucine, proline, serine, thiamine and valine were found to be favorable for the PME production by both isolates. However, glycine, histidine and aspartic acid were found to be better for isolate S 15-5 and isolate S 15-16 in the production of PME. Methionine is better for PME production by isolate S 15-16 than isolate S 15-5. PG production was favored by alanine, cystine, and glycine. Glutamine, leucine, lysine HCl, methionine and valine were of no use for PG production by both isolates. However, from the data given in Table 5, it showed that the use of amino acid for the production of enzymes by these two isolates was not quite similar to each other.

Discussion and Conclusion

Bateman (1963) reported that pectolytic enzyme was associated with bean hypocotyle infected with *Rhizoctonia solani*. Data obtained from the present investigation give evidence that the production of PG by the causal fungus is responsible for the pathogenic mechanism on leaf blight of soybean. Although PME always existed both in tissue extracts and cultures filtrates, no significant correlation was found to disease development. From this point of view, PG is important in tissue degradation.

Table 5. Effects of amino acids on relative activity of PME and PG produced by *T. cucumeris* in liquid culture incubated for 7 days at 30°C.

Amino acids.	Isolates S 15-5		Isolates S 15-16	
	PME[1]	PG[2]	PME	PG
DL-Alanine	0.37	48.97	0.27	34.78
L-Arginine	0.38	0	0	50.00
L-Asparagine	0.47	34.00	0.19	14.00
DL-Aspartic acid	1.68	12.16	0.78	50.00
L-Cystine	0.72	33.30	0.26	34.88
L-Glutamic acid	1.50	6.50	1.60	33.96
L-Glutamine	1.59	0	1.23	0
Glycine	1.67	35.00	0.60	74.00
L-Histidine	1.04	0	0.22	12.00
DL-Isoleucine	1.44	11.53	1.43	0
L-Leucine	1.43	0	1.48	0
L-Lysine HCl	1.40	0	0.74	0
L-Methionine	0.80	0	1.57	0
DL-Phenylalanine	1.40	10.86	0.54	65.11
L-Proline	1.15	13.04	1.39	0
DL-Serine	1.13	0	1.40	12.50
DL-Threonine	1.03	63.88	1.49	0
DL-Valine	1.09	0	1.60	0

1. 2. See Table 1.

Generally, high virulent isolates produced more amount of PG, but some isolates of high virulence on soybean leaves produced no more amount of PG than others. This indicated other factors, as enzymes combine with PG to macerate the leaf tissues, were involved. van Etten *et al.* (1967) reported the cellulase was responsible in the lesion formation in bean hypocotyle tissue infected by *R. solani*. It is no doubt that the pathogenicity of *T. cucumeris* is at least in part due to the action of pectic enzyme particularly PG produced by the fungus.

Previous report (Han, 1966) pointed out that rapid development of leaf blight occurred at the temperature between 28~30°C. This temperature also is the conditions favorable for mycelial growth and pectic enzyme activities. No enzyme was detected from the culture filtrates at pH above 8.5, although the mycelial growth did occur. This might be due to the inactivation of the enzyme at the higher pH as described by Batemen (1963). Data obtained from the effects of nutritional sources on pectic enzyme activities which showed that the utilization of nutrients for mycelial growth is in general coincidence with pectic enzyme activities. The cultural and nutritional conditions are main factors affecting the production of pectic enzymes in liquid culture. Pectic substances are known nutritional material for production of pectic enzymes in culture. Present studies also indicated that nitrogen sources were

important factors in association with the amount of pectic enzyme produced in liquid culture. The utilization of nutritional sources by different isolates is quite different. The differences in pathogenicity of the isolates might de due to the difference in nutritional requirement for pectic enzyme activities. On the other hand, the reason why certain soybean varieties are extremely susceptible to infection by the causal fungus, whereas others are resistant may satisfactorily be explained that there might be some nutritional factors in soybean which may inhibit or stimulate the pectic enzyme activities. An artificial medium special for enzyme production by the test fungus could be designed according to the data obtained from the present investigation.

Summary

Pectic enzyme activities of *T. cucumeris*, the soybean leaf blight fungus, was studied both in soybean leaf-tissues and in culture filtrates. *T. cucumeris* is capable of producing two pectic enzymes, pectin methylesterase (PME) and polygalacturonase (PG). Results obtained from enzymatic assay with soybean leaf-tissus showed that higher amount of PG was found in diseased tissues than in healthy tissues. The pathogenic isolates of *T. cucumeris* are potentially higher in PG production in liquid culture. The pathogenesis of soybean leaf blight (aerial blight) is at least in part due to the action of pectic enzyme particularly the PG secreted by the causal fungus during infection. From the current studies it is clear that PME may be of secondary importance in pathogenesis and is perhaps not functionally associated with host tissue degradation. 28~32°C is the optimum temperature range favorable for pectic enzyme production. Optimum pH for enzyme production in liquid culture is 5.0~5.5. The activities of PME and PG in Czapek's solution differed according to the isolates, cultural conditions, and nutritional sources used. Relatively higher amount of PME was detected in cultures containing pectic acid, xylose, rhamnose and lactose as carbon source. Pectic acid, arabinose, rhamnose, soluble starch were found to be favorable for PG production in liquid culture. Ammonium sulphate and ammonium tartrate were favorable for the production of PME and PG. Sodium nitrate, potassium nitrate, glycocoll were found to be best for PG production. Among amino acids tested, alanine, cystine and glycine gave similar results for PG production. Aspartic acid, glutamic acid, glutamine, isoleucine, leucine, serine, threonine and valine are good substrate for the production of PME.

Literature Cited

Barker, K. R. and J. C. Walker. 1962: Factors affecting the pathogenicity of *Pellicularia filamentosa*. Phytopathology 52: 2.

Barker, K. R. and J. C. Walker. 1962. Relationship of pectolytic and cellulytic enzyme production of strains of *Pellicularia filamentosa* to their pathogenicity. Phytopathology 52: 1119~1125.

Bateman, D. F. 1962. Pectic enzymes associated with Rhizoctonia-infected tissues of bean. Phytopathology 52: 2.

Bateman, D. F. 1963. Pectolytic enzyme activities of culture filtrates of ***Rhizoctonia solani*** and extracts of Rhizoctonia-infected tissues of bean. Phytopathology 53: 197~204.

Bateman, D. F. 1964. Cellulase and the ***Rhizoctonia*** disease of bean. Phytopathology 54: 1372~1377.

Bateman, D. F. and D. P. Maxwell. 1965. Phenol oxidase activity in extracts by ***Rhizoctonia solani***-infected hypocotyls of bean and its distribution in relation to lesion areas (abstr.) Phytopathology 55: 127.

Boosalis, M. G. 1947. Necrosis of soybean stem and root caused by ***Rhizoctonia solani*** (abstr.) Phytopathology 37: 3.

Boosalis, M. G. 1950. Studies on the parasitism of ***Rhizoctonia solani*** Kuhn on soybeans. Phytopathology 40: 820~831.

Chen, C. C. 1963. Sclerotial diseases of rice plant in Taiwan Mem. College Agr., Natl. Taiwan University 7: 37~54.

Chen, D. W., T. C. Lo, and C. C. Lee. 1963. Studies on the damping-off of china-fir (***Cunninghamia lanceolata*** H.). Jour. of Agriculture and Forestry 12: 279~323.

van Etteen, H. D., D. P. Maxwell, and D. F. Bateman. 1967. Lesion maturation, fungal development, and distribution of endopolygalacturonase and cellulase in Rhizoctonia-infected bean hypocotyl tissues. Phytopathology 57: 121~126.

Flentje, N.T. and. D. J. Hagedorn. 1964. Rhizoctonia tip blight and stem rot of pea. Phytopathology 54: 788~791.

Fukumoto, J. 1956. Pectic enzyme. In Akabori, S.: Methods in enzymology, Asakura book Co. Vol. 2. pp. 163~171 (In Japanese).

Garrett, S. D. 1962. Decomposition of cellulose in soil by ***Rhizoctonia solani*** kuhn. Trans. Brit. Mycol. Soc. 45: 115~120.

Gonzalez, L. C. and J. H. Dwen. 1963. Soil rot of tomato caused by ***Rhizoctonia solani***. Phytopathology 53: 82~85.

Han, Y. H. 1966. Banded sclerotial blight of soybean caused by ***Pellicularia sasakii*** (shirai) Ito in Taiwan. Plant Prot. Bull. 8: 101~118.

Kertesz, Z. I. 1955. Pectic enzymes. In Sidney P. Colowick and Nathan O. Kaplan: Methods in enzymology. Academic Press, New York. Vol. 1. pp. 158~166.

Lai, M. T. and L. C. Wu. 1963. Some observations on the infection of mung bean seedling by ***Rhizoctonia solani*** Kuhn. Plant Protection Bulletin 5: 386~397.

Maxwell, D. P. and D. F. Bateman. 1967. Changes in the activities of some oxidases in extrates of Rhizoctonia-infected bean hypocotyls in relation to lesion maturation. Phytopathology 57: 132~136.

Mann, B. 1962. Role of pectic enzymes in the ***Fusarium*** wilt syndrome of tomato. Trans. Brit. Mycol. Soc. 45: 169~178.

Nakayama, T. A. 1940. A study of the infection of cotton seedlings by ***Rhizoctonia solani***. Ann. Phytopathology Soc. Japan 10: 223.

Newton W. and N. Mayers. 1935. The physiology of ***Rhizoctonia solani*** kuhn. IV. The effect of a toxic substance produced by ***Rhizoctonia solani*** kuhn when grown in liquid culture, on the growth of wheat, carrots, and turnips. Sci. Agr. 15: 399 ~401.

Ryker, T. C. and F. S. Gooch. 1938. ***Rhizoctonia*** sheath spot of rice. Phytopathology 28: 233~246.

Sakesona, H. K. and O. Vaartaja. 1961. Taxonomy, morphology, and pathogenicity of ***Rhizoctonia*** species from forest nurseries. Canadian Jour. of Botany 39: 627.

Sherwood, R. T. and C. G. Lindberg. 1962. Production of a phytotoxin by ***Rhizoctonia solani***. Phytopathology 52: 586~578.

Smith, W. K. 1958. A survey of the production of pectic enzymes by plant pathogenic and other bacteria. J. Gen. Microbiol. 18: 33~41.

Tweedy, B. G. and D. Poweel. 1962. Production of pectin-polygalacturonase by ***Alternaria mali*** (abstr.). Phytopathology 52: 30~31

Woodbury, W., C. C. Chi, and E. W. Hanson. 1962. Pectic enzyme production by three ***Fusarium*** spp. from red clover. (abstr.) Phytopathology 52: 33.

Wu, L. C. 1965. Physiology of parasitism. 1. Growth pathogenicity, and toxin production of ***Rhizoctonia solani*** kuhn. Botanical Bulletin of Academia Sinica 5: 144~152.

Wu, L. C. and V. S. Lin. 1967. Rhizoctonia aerial blight of soybean by ***Thanatephorus cucumeris***. Memoirs of the college of Agriculture National Taiwan University, Vol. 9: 57~69.

Wyllie, T. D. 1962. Effect of metabolic by-produtcs of ***Rhizoctonia solani*** on the roots of chippewa soybean seedlings. Phytopathology 52: 202~206.

大豆菌核病菌之果膠分解酵素及影響酵素活性之因子

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摘 要

本文乃報告 *Thanatephoruis cucumeris* (Frank) Donk 於大豆罹病組織中果膠分解酵素之特性及影響酵素活性之因子。本病原菌於大豆葉片罹病組織及液體培養濾液中有 Pectin methylesterase (PME)及 Polygalacturanase (PG)兩種果膠分解酵素之產生。大豆罹病葉片組織中 PG 之含量較健全組織所含有者多達二至十倍，而健全組織及罹病組織中 PME 之含量均微少且無顯著差異。病原性較強之菌株於罹病組織及培養濾夜中之 PG 生成量較病原性弱者爲多。本病原菌之病原性與 PG 之作用有密切關係，而 PME 與病相 (Pathogenesis) 無密切關連。菌絲生長之最適溫度亦爲果膠分解酵素產生之適宜溫度，尤以 PG 最爲明顯。培養基之 pH 除直接影響菌絲之生長外亦影響果膠分解酵素之產生。pH 5.0～5.5 對 PG 及 PME 之產生最爲適宜，pH 3.5 以下及 8.0 以上酵素生成量極微。病原菌菌絲生長所需營養源與 PG 和 PME 產生所需者相近似。對 PG 及 PME 之生成而言其所需之碳氮素源略有不同，菌株間亦略有差異。碳素源中以 pectic acid, sorbital, sorbose, xylose, rhamnose 及 lactose 對 PME 之產生最爲適宜。pectic acid, arabinose, rhamnose 及 soluble starch 對 PG 之產生則較爲適宜。氮素源中之 sodium nitrate, potassium nitrate 及 *glycocoll* 對 PG 之生成最屬重要。氨基酸中以 alanine, arginine, asparagine, cystine, glutamic acid, phenylalanine 及 glycine 對 PG 生成之需求最爲明顯，兩菌株亦顯有不同。由本研究結果可知培養條件與病原菌果膠分解酵素之活性有密切關係。