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人蔘뿌리썩음病菌, *Cylindrocarpon destructans*에 의한 纖維素分解酵素 및 펙틴質分解酵素의 分泌 및 抑制*

李 震 雨**·鄭 厚 燮**

Production and Inhibition of Cellulolytic and Pectolytic Enzymes by *Cylindrocarpon destructans*(Zins.) Scholten Causing Root Rot of Ginseng*

Jin Woo Lee** and Hoo Sup Chung**

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Abstract

The activities of pectolytic and cellulolytic enzymes produced from slices of ginseng root infected with *Cylindrocarpon destructans*(Zins.) Scholten were proportional to each concentration and reaction time. Activities of cellulase(Cx), endo-polygalacturonase(endo-PG), endo-polymethylgalacturonase(endo-PMG), exo-polygalacturonase(exo-PG), and exo-polymethylgalacturonase(exo-PMG) were maximum on the 4th day after inoculation. No endo-PG and endo-PMG were detected at the first and second days, while exo-PG exo-PMG were active. On the 6th day, all pectic enzymes were completely lost, whereas Cx remained at a high concentration. pH optima of Cx, endo-PG, endo-PMG, exo-PG, and exo-PMG were 6.0, 5.5, 8.0, 7.0 to 7.5, and 8.5, respectively. Temperature optima of Cx, endo-PG, endo-PMG, exo-PG, and exo-PMG were 66°C, 53°C, 41°C, 37°C, and 40°C, respectively. Cx was only inhibited by 0.05M Hg^{++} among 16 ions tested. Inhibitory effects of ions on pectolytic enzymes varied, however 0.005M Fe^{+++} and 0.05M Al^{+++} were the best in general. Among 8 fungicides, none of them inhibited all the enzymes studied at 0.1% active ingredients. Exo-PG were highly inhibited by all of the fungicides, of which difolatan was the most inhibitory to all the pectic enzymes. Ca^{++} at 0.2M and Fe^{+++} at 0.02M completely inhibited all the pectolytic enzymes, and Cx was inhibited 30% and 70% at the same concentration, respectively. Formalin almost inhibited exo-PG and exo-PMG at 0.8% but not the other enzymes especially Cx. Difolatan at 0.8% inhibited all the enzymes concerned above 80%. The cellulolytic and pectolytic enzymes of *C. destructans* must be closely associated with the ginseng root rot and should be inhibited to control the disease effectively.

Introduction

Korean ginseng(*Panax ginseng* Meyer.), famous for a medical herb has increased its demand in Korea exporting more than $10 million worth of the ginseng products to foreign countries. However there are a

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**: Department of Agricultural Biology, College of Agriculture, Seoul National University Suwon, Korea. Present address of the senior author: Department of Plant Pathology, Institute of Agricultural Science, Office of Rural Development, Suwon, Korea. (서울大學校 農科大學 農生物學科 韓國 水原, 先著者의 現住所 : 農村振興廳 農業技術硏究所 病理科 韓國 水原).

lot of problems to be solved for increasing the yield and expansion of the cultivation. It is believed that the field cultivated for the crop once has to be rotated more than 10 years due to soil-borne diseases, especially ginseng root rot caused by *Cylindrocarpon destructans*(Zins.) Scholten. The losses incurred by various ginseng root diseases were 25% at Yangji and 30% at Kangwha[13]. In 1965 bacterial root rot occurred about 48% at Keumsan and as much as 75% at Puyu[18].

Since ginseng grows in fertile soil with a rich humus in the shade for six years until harvest, there are no practical control measures for the soil-borne diseases. Fungicidal application to the soil may not be feasible to control the disease even when the fungicides are effective, because of the phytotoxicity as well as residual problems. It has been reported, however, that soil amendments of mineral nutrients might reduce the incidence of soil-borne diseases. When cotton plants inoculated with *Fusarium vasinfectum* were grown in soil amended with Zn^{++}, pectin methylesterase(PME) activity was retarded, and this was perhaps due to the in-vivo inhibition of the enzyme[29]. The role of enzyme inhibitors with mineral amendments in the ginseng field would be a worthwhile approach for practical control of root rot.

It is well known that roles of pectolytic and cellulolytic enzymes responsible for the tissue degradation in infected plants have been reviewed extensively by both Bateman and Millar[4], and Wood[37]. Among the pectolytic enzymes, endo-polygalacturonase(endo-PG) seemed to be the main contributor to the maceration of host tissues due to their functions[1,8,9,25,33,34]. Cellulolytic enzymes in relation to the tissue degradation have also been studied with various diseases [1,5,15,32]. However, nothing is known about the pectolytic and cellulolytic enzymes associated with *C. destructans* causing severe epidemics of ginseng root Mil'ko and Mel'nik[23] only detected the presence of PG and PME from *C. radicicola*.

In general, Hg^{++}, Co^{++}, Cr^{++} and Pb^{++} inhibited cellulases according to recent review by Mandels and Reese[21]. Of the inhibitors investigated Hg^{++} was widely respresented the most active inhibitor of cellulases[12,15,30]. PG was also known to be inhibited by the Ca^{++}[4,24,31,32]. Corden et al[11] discussed that Fe^{+++}, Al^{+++}, Ag^{++}, Cu^{++}, Co^{++}, Ni^{++} and Cr^{++} inhibited PG more than the Ca^{++}. On the other hand, among fungicides, ferbam, nabam, and ziram partially inhibited PG secreted by *Cladosporium cucumerium*[15]. Predominant natural inhibitors of pectolytic and cellulolytic enzymes were phenolic compounds, substances derived from them after oxidation[27,28] or tanins[32]. While inhibitors of pectolytic and cellulolytic enzymes were known, the extensive investigation of the enzyme inhibitors with *C. destructans* would afford a basic information for the control measures in the field.

The purposes of the present study were to detect pectolytic and cellulolytic enzymes produced by *C. destructans*, to investigate some characteristics of the enzymes concerned and to look into the role of the inhibition by ions and fungicides for controlling the disease.

The authors deeply express their sincere gratitude to Dr. Su Rae Lee, the Korea Atomic Energy Research Institute in rendering valuable advices in this experiment and to the Kwachon Ginseng Experiment Station, Office of Monopoly for some ginseng roots obtained. The assistance of Mr. Jeong Hwa Kim in providing figures is gratefully acknowledged.

Materials and methods

Isolate used

A virulent isolate of *Cylindrocapon destructans* (Zins.) Scholten from the lesions of the root rot of 3-year old ginseng grown at Ginseng Experiment Station in Kwachon, Kyunggi-do was used throughout this investigation. The stock cultures on potato sucrose agar(PSA) were maintained at 3°C to 5°C.

Enzyme preparations

Crude enzyme preparations were obtained from the 4-day old cultures grown on the slices of fresh ginseng root in the most of this study. The 4-year old roots of about 1.5 to 2 cm in diameter were chopped into slices of 1cm in thickness. The slices thus made were disinfected with 0.1% NaC10 solution for 3 min and rinsed with sterile water several times. The

slices of 30g were wounded with a needle and then inoculated with about 10ml of the conidial suspension (1.5×10^5 conidia/ml) prior to keeping them in a petri plate in which a moistened filter paper was placed.

For the inhibition study, the culture was grown for 7 days on the wheat-bran medium consisting of 10g of wheat-bran and 10ml of distilled water in a 500ml Erlenmeyer flask. The medium was autoclaved at 121°C for 20min. The cultures were kept in an incubator at 28±1°C.

After the growth period, the cultures both on the slices and on the wheat-bran medium were ground in a Waring blendor containing 100ml of 0.005M acetate buffer(pH4.5). The homogenizents, added with several drops of toluol, were kept at 5°C for an hour. The pulp and hard chaff were then removed by squeezing the liquid through several layers of cheese-cloth. The liquid fractions were centrifuged at 3,000 rpm for 20 min to get the clean crude enzyme preparations. The enzyme preparations thus obtained from the slices were dialyzed against distilled water for about 24 hr at 5°C. The enzyme preparations were stored at 5°C until used.

Measurement of enzyme activities

In a preliminary experiment, the relative activity (RA) for each enzyme had been proved to be proportional to enzyme concentration and reaction time (Fig.1 and 2).

All experiments were performed with 3 replications at 30 C in a water bath unless otherwise indicated. Each of the 0.5% solutions of the enzyme assay; Carboxylmethylcellulose(CMC) for cellulase(Cx), Pectic acid(Carnoy Product Co. N.Y.) for endo-PG, and exo-PG and Pectin N.F.(Carnoy Product Co. N.Y.) for endo-PMG and exo-PMG.

1. Cellulase, endo-ploygalacturonase and endo-polymethylgalacturonase

Enzyme activities of Cx, endo-PG and endo-PMG were measured by modified method of Lee et al's[19] using the Ostwald-Fenske viscosimeter containing total reaction mixture of 3 ml of each substrate and 2 ml of the enzyme preparation except otherwise specifically described. The activities of the enzymes

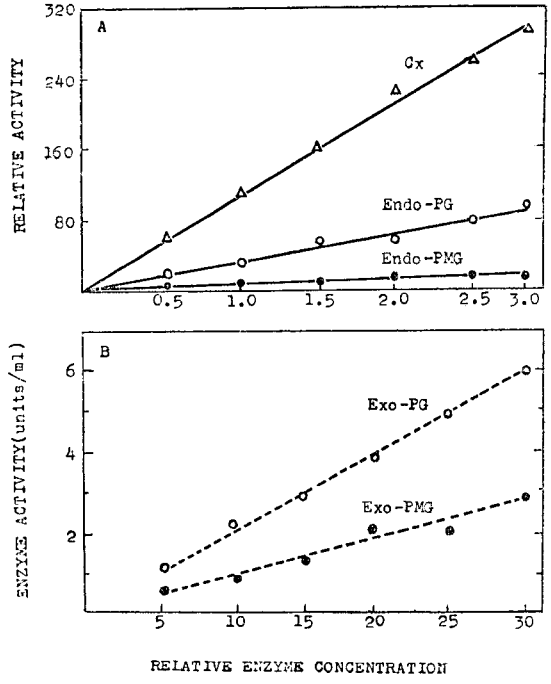

Fig. 1. Effect of enzyme concentration on activities of A) cellulase(Cx), endo-polygalacturonase(endo-PG) and endo-polymethylgalacturonase(endo-PMG), and B) exo-polygalacturonase(exo-PG) and exo-poly methylgalacturonase(exo-PMG) obtained from the slices of ginseng root infected with *Cylindrocarpon destructans*

were expressed in terms of relative activity; RA was defined as 1,000 multiplied by the reciprocal time in minutes necessary for a 50% loss in viscosity for cellulase or 20% for the other enzymes.

2. Exo-polygalacturonase and exo-polymethyl galacturonase

The activities of exo-PG and exo-PMG were determined by measuring reduced groups by Jansen and MacDonell's modified method originated from the hypoiodite method of Willstäter-Schudel[17]. The reaction mixture contained 10% crude enzyme preparation and the final concentration of pectic acid or pectin was 0.045%. At regular time intervals, I_2 liberated was titrated with 0.1N $Na_2S_2O_3$ using starch as an indicator. The activity was expressed in units per ml of the preparation, a unit being the amount of the enzyme required to liberate microequivalent reducing groups in one min in a 5ml aliquot from the reaction mixture.

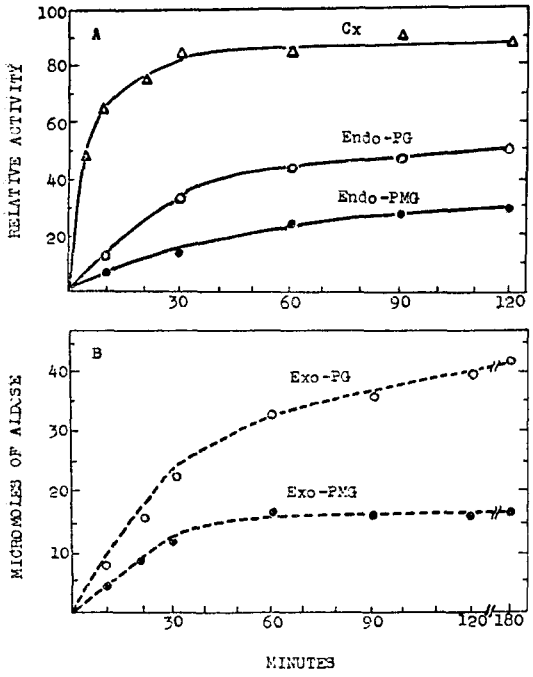

Fig. 2. Effect of reaction time on activities of A) cellulase(Cx), endo-polygalacturonase(endo-PG) and endo-polymethylgalacturonase(endo-PMG), and B) exo-polygalacturonase(exo-PG) and exo-polymethylgalacturonase(exo-PMG) obtained from the slices of ginseng root infected with *Cylindrocarpon destructans*

Inhibition by ions and fungicides

Unless otherwise indicated, each fungicide diluted to 0.1% solution on the basis of active ingredients and ions diluted with to 0.05M solution except 0.005M Fe^{+++} and were then used. Hg^{++}, Ca^{++}, Cu^{++}, Co^{++}, Li^{+}, Mg^{++}, Sn^{++}, and Sr^{++} were furnished as the chlorides, while Fe^{+++}, Ba^{++}, NH^{4+}, Al^{+++}, Ni^{++}, K^{+}, Na^{+} and Zn^{++} were used as the sulfates. The following fungicides were tested; zineb(zinc ethylene-bis-dithiocarbamate 70%), maneb(manganese ethylene-bis-dithiocarbamate 70%), difolatan(cis-N-[1.1.2.2-tetra chloroethyl] thio-4-cyclohexane-1.2-di-carboximide 20%), captan(n-trichloro-methylthio 1.2.3.6-tetrahydrophthalimide 50%), PCNB(pentachloronitrobenzene 20%), vitavax(2.3-dihydro 5-carboxoxanilido-6-methyl-1.4-oxanthiin 25%), benlate(benomyl [methly-1 buthly cabamoyl]-2 benzimibuzol carbamate 50%) and formalin(formaldehyde 37%).

Cheks were run with thee same volume of distilled water instead of inhibitors. The inhibition studies were conducted with a modification of the Husain and Rich's method[16]. The enzyme preparations were allowed to remain in contact with an equal amount of inhibitors for one hour prior to the addition of each substrate. All procedures were the same as the prescribed previously except using the equal volume of each enzyme-inhibitor complex instead of the crude enzyme preparations.

Results

Activities change according to days after inoculation

Cellulolytic and pectolytic enzyme activities were not detected in the extracts from the noninoculated ginseng roots. All of the pectolytic enzymes were most active on the 4th day after inoculation. The enzyme activities of both endo-and exo-forms were none on the first and the second days, whereas the exo-forms

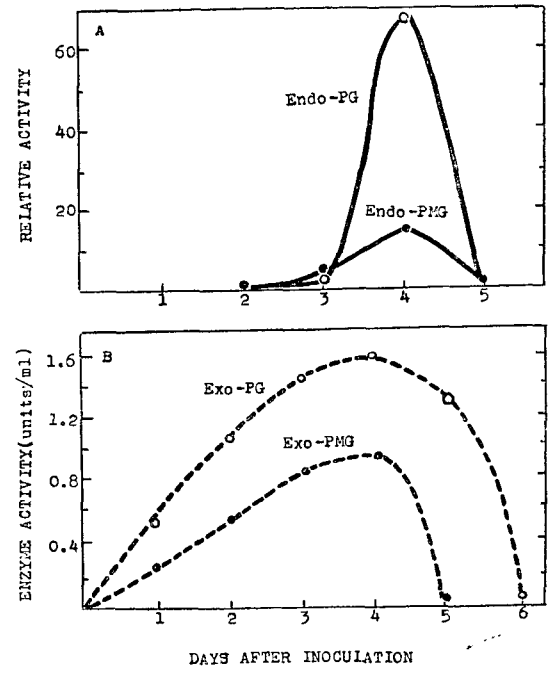

Fig. 3. Changes in activities of A) endo-poly-galacturonase(endo-PG) and endo-polymethylgalacturonase(endo-PMG), and B) exo-polygalacturonase (exo-PG) and exo-polymethylgalacturonase(exo-PMG) when the slices of ginseng root were inoculated with *Cylindrocarpon destractans*

gradually increased their activities from the first day after inoculation. The activities of pectolytic enzymes were abruptly dropped on the 5th day except that of exo-PG which gradually decreased on the 6th day (Fig. 3). The activity of endo-PG was more than 4 times that of endo-PMG at the same concetration. Cx activity was always the highest among all the 5 enzymes studied, gradually increasing from the first day and peaking on the 4th day after inoculation and thereafter decreasing slowly (Fig. 4).

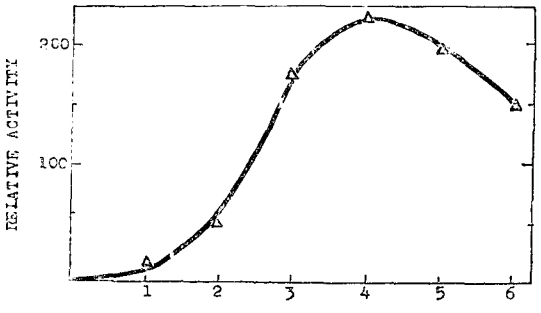

Fig. 4. Changes in activity of cellulase(Cx) when the slices of ginseng root were inoculated with *Cylindrocaron destructans*

Effect of pH and temperature

To determine the effect of pH on the enzyme activities of the exo-PG and exo-PMG, each of the substrates was adjusted to various pH levels with acetate buffers for those below pH 7.0 and with boric acid borax buffers for those above pH 7.0. For Cx, endo-PG, and endo-PMG, the aliquotes of freshly prepared 1.5% each substrate in 0.05M acetate buffers (pH 4.5) were adjusted with 0.1N NaOH or 0.1N HCl to the desired pH level. Distilled water was added to give the substrate concentration of 0.5% before 2ml of the enzyme preparations was combined to a viscosimeter.

The optimum pH ranges of Cx were from 5.0 to 7.0 centering at 6.0. The activity of Cx increased rapidly in acidic ranges of up to pH 6.0, then decreased gradually in alkalic ranges. Endo-PG did not react at pH 2.5, but then abruptly increased from this point up to 5.5, then decreased rapidly in the higher pH ranges. Endo-PMG was considerably less active than those of Cx and endo-PG. The pH optima of exo-PG and exo-PMG were 7.0 to 8.0 and 7.5 to 9.5, respectively (Fig. 5). The activity of exo-PG was

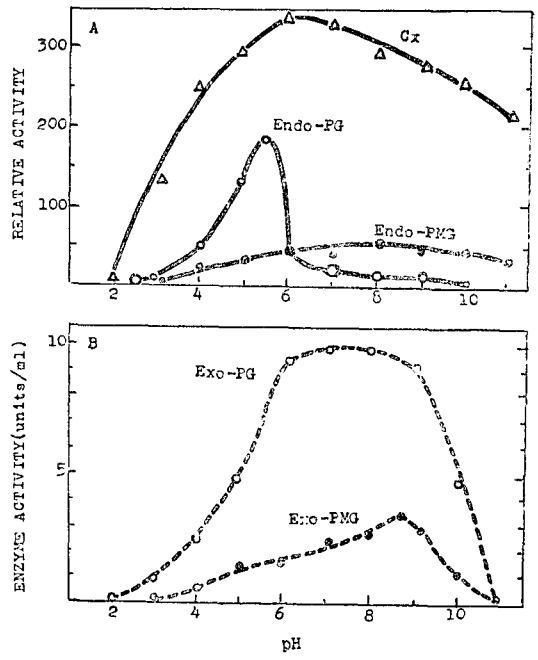

Fig. 5. Effect of pH on activities of A) cellulase (Cx), endo-polygalacturonase (endo-PG) and endo-polymethylgalcturonase (endo-PMG), and B) exo-polygalacturonase (exo-PG) and exo-polymethylgalacturonase (exo-PMG) obtained from the slices of ginseng root inoculated with *Cylindrocaron destructans*

more active than endo-PMG below pH 6.0.

Effect of temperature was conducted in a water bath at given temperatures. Cx, endo-PG and endo-PMG had different optimum temperature ranges. Cx activity gradually increased as the temperature increased up to 66 C, then suddenly decreased to almost nothing at 80 C. The activity of endo-PMG did not increase significantly at first. It gradually increased latter until 53°C, then completely stopped at 60°C. The activity of endo-PG gradually increased as the temperature increased until 41°C and then stopped at 45°C. The optimum temperatures of exo-PG and exo-PMG were 37°C and 40°C, respectively. Exo-PMG was affected, considerably less than that of exo-PG as a whole.

Inhibition by ions and fungicides

1. Screening inhibitors

To find out whether ions and fungicides inhibit the enzyme of *C. destructans*, the enzyme preparations

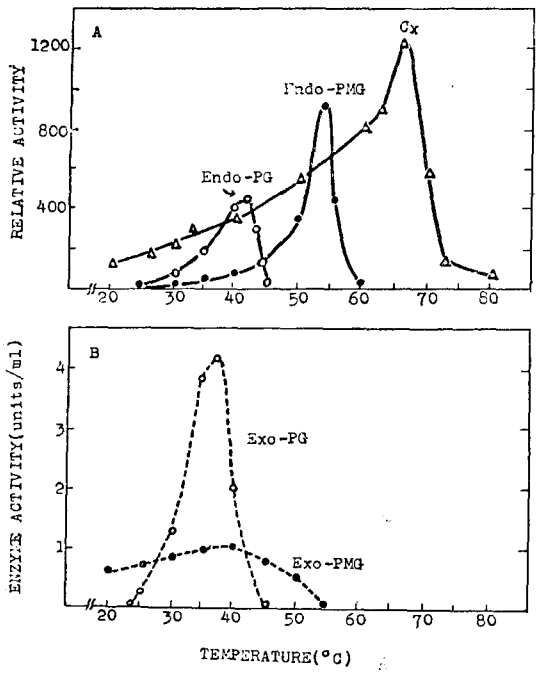

Fig. 6. Effect of temperature on activities of A) cellulase(Cx), endo-polygalacturonase(endo-PG) and endo-polymethylgalacturonase(endo-PMG), and B) exo-polygalacturonase(exo-PG) and exo-polymethylgalacturonase(exo-PMG) obtained from the slices of ginseng root inoculated with *Cylindrocarpon destructans*

obtained from the wheat-bran medium were mixed with an equal volume of the inhibitors. Enzyme assays were made as previous methods in a water bath at 35°C.

Among 16 ions, Hg^{++} solely inhibited the activity of Cx up to 63%. Endo-PG was highly inhibited by Fe^{+++}, and then followed by Sn^{++}, Sr^{++}, Hg^{++}, and Zn^{++}. Cu^{++}, Co^{++}, Li^{+}, Mg^{++}, Ba^{++}, and K^{+} had no affect. Endo-PMG was highly inhibited by Fe^{+++}, Al^{+++}, NH_4^{+} and Zn^{++}; moderately by Ni^{++}, Hg^{++}, Ba^{++}, Cu^{++}, Sr^{++}, Na^{+}, Co^{++}, Li^{+} and K^{+}; no inhibitory or only meager affects by Ca^{++}, Mg^{++} and Sn^{++}. Exo-PG was highly inhibited by Mg^{++}, Na^{+}, Fe^{+++}, Sr^{++}, Co^{++}, Al^{+++} and Ni^{++}; other ions except K^{+} were moderate inhibitors. Exo-PMG was completely inhibited by Fe^{+++}; strongly inhibited by Mg^{++}, NH_4^{+} and Hg^{++}, while Al^{+++}, Ni^{++}, Na^{+}, Ca^{++} and K^{+} were moderate inhibitors on this enzymes (Table 1). Most of the ions studied specifically inhibited one or more enzymes to some extent, whereas Hg^{++} was highly inhibitory to Cx as well as the pectolytic enzymes. Among the ions tested, Fe^{+++} and Al^{+++} were highly inhibitory to the pectolytic enzymes. There were no differences in inhibitions among the mono-, di-, and tri-valent ions.

Table 1. Effect of ions on the celluloytic and pectolytic enzymes obtained from the wheat-bran medium inoculated with *Cylindrocarpon destructans*

Ions[b]	Percent inhibition[a]				
	Cx	Endo-PG	Endo-PMG	Exo-PG	Exo-PMG
Hg^{++}	63	43w	32w	60v	59x
Ca^{++}	0	19u	2v	36t	36v
Cu^{++}	0	0	31w	58v	0
Co^{++}	0	0	19w	68wx	14u
Li^{+}	0	0	19w	45u	0
Mg^{++}	0	0	0	100z	82y
Sr^{++}	0	44w	20w	74wxy	0
Sn^{++}	0	64x	2v	48uv	0
Fe^{+++}	0	95z	74z	77xy	100z
Ba^{++}	0	0	31w	48uv	27u
$NH4^{+}$	0	12t	48x	36t	73y
Al^{+++}	0	81y	56y	61vw	46w
Ni^{++}	0	18u	32w	61vw	46vw
K^{+}	0	0	17w	3s	36v
Na^{+}	0	3t	20w	81y	43w
Zn^{++}	0	33v	37x	58v	0

a. An average of 3 replicates. Numbers followed by the same letters are not significantly different (P=0.99).

b. Hg^{++}, Ca^{++}, Cu^{++}, Co^{++}, Li^{+}, Mg^{++}, Sr^{++}, and Sn^{++} are furnished as the chlorides, while Fe^{+++}, Ba^{++}, NH_4^{+}, Al^{+++}, Ni^{++}, K^{+}, Na^{+} and Zn^{++} as the sulfates.

Cx activity was slightly inhibited by zineb and vitavax, while the other enzymes had little or no affect. Endo-PG activity was partially inhibited by formalin, benlate, difolatan, captan and maneb, in the above order. None were inhibitory to the activity of endo-PMG except difolatan. Others had only meager affects. All of the fungicides tested considerably inhibited the exo-PG activity. The most effective one was formalin. Exo-PMG activity was inhibited by all of the fungicides except formalin. The most inhibitory was maneb; difolatan, captan, PCNB, benlate, zineb, and vitavax were next in order(Table 2). Difolatan effectively inhibited the 4 pectolytic enzymes. In general exo-form enzymes of the pectolytic enzymes were more inhibited by the fungicides than were the endo-forms.

Table 2. Effect of fungicides on the cellulolytic and pectolyic enzymes obtained from the wheat-bran medium inoculated with *Cylindrocarpon destructans*

Fungicides[b]	Per cent inhibition[a]				
	Cx	Endo-PG	Endo-PMG	Exo-PG	Exo-PMG
Zineb	25 y	0 x	0	63 x	9 w
Maneb	0	18 x	0	54 w x	83 z
Difolatan	4 x	27 y	26 y	42 w	61 y
Captan	1 x	19 x	0	67 y	39 x
PCNB	0	0	0	57 x	39 x
Vitavax	21 y	0	1 x	51 w	4 w
Benlate	0	28 y	0	54 w x	39 x
Formalin	3 x	29 y	2 x	80 z	0

a. An average of 3 replicates. Numbers followed by the same letter are not significantly different ($P=0.99$).

b. Based on 0.1% of active ingredients.

2. Effect of inhibitor concentration

The inhibitory effects of Ca^{++}, Fe^{+++}, formalin, and difolatan screened in the precious study were tested further with various concentrations against the enzyme activities.

Exo-PG and endo-PG were completely inhibited at 0.1M Ca^{++}, while the same thing happened for exo-PMG and endo-PMG with 0.2M Ca^{++}. Inhibition of Cx occurred at a level of only 33% at 0.2M Ca^{++}. Most of the pectolytic enzyme activities were inhibited more than 80% by 0.01M Fe^{+++}, but Cx activity was not inhibited at all at same concentration. At 0.02M Fe^{+++}, the Cx activity was decreased to 66% (Fig. 7). Cx activity was hardly inhibited by Ca^{++}, but the concentration of Fe^{+++} which contained onlyea tenth of that of Ca^{++} inhibited the enzyme more than Ca^{++}.

Exo-PG and endo-PMG were inhibited by 0.4% of formalin to about 90%. The activities of Cx, endo-PG, and exo-PMG were inhibited about 20, 55, and 25% using 0.4% formalin, respectively. At 0.4 to 0.5% of formalin, all enzymes were inhibited almost evenly. Endo-PG was completely inhibited by 0.4% of difolatan. Other pectolytic enzymes resulted in about 70 to 90% inhibition with the same concentration except

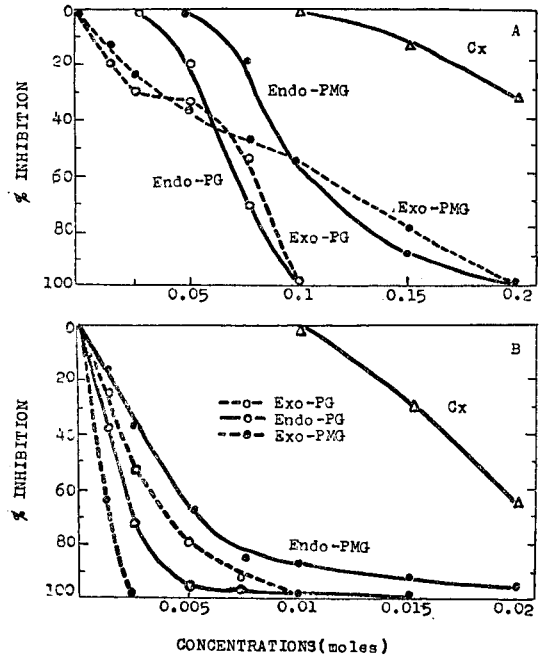

Fig. 7. Effect of the concentrations of A) Ca ion and B) Fe ion on cellulase(Cx), endo-polygalacturonase(endo-PG), endo-polymethylgalcturonase(endo-PMG), exo-polygalacturonase(exo-PG) and exo-polymethylgalacturonase(exo-pMG) obtained from the slices of ginseng root inoculated with *Cylindrocarpon destructans*

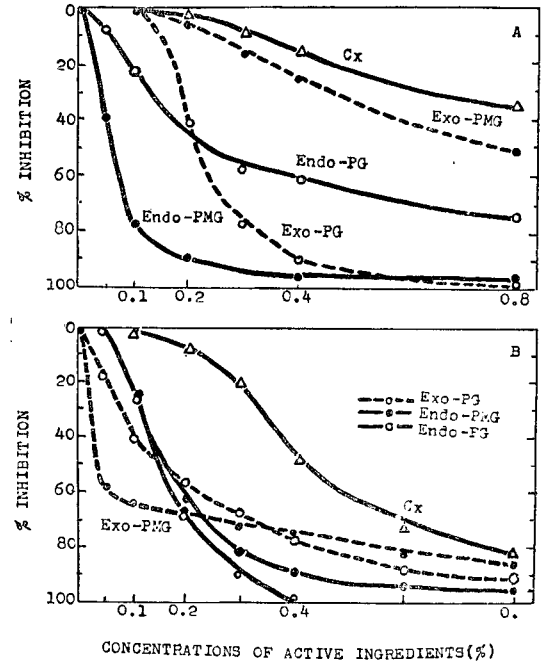

Fig. 8. Effect of the concentration of A) formalin and B) difolatan on cellulase(Cx), endo-polygalacturonase(endo-PG), endo-polymethylgalacturonase(endo-PMG), exo-polygalacturonase(exo-PG) and exo-polymethylgalacturonase(exo-PMG) obtained from the slices of ginseng root inoculated with *Cylindrocarpon destructans*

Cx which was inhibited about 50%. All five enzymes including Cx were inhibited about 80% at 0.8% of difolatan(Fig. 8).

Discussion

Cx activity of *C. destructans* was not only higher in its action but also more stable in wide ranges of pH and temperatures than pectolytic enzymes throughout this study. Moreover Cx activity was not readily extinguished at the time when all pectolytic enzymes were completely inactivated. It seems probable that cellulase might faciliate to penetrate the ginseng root by degradating the polymerized cell walls as well as to cause the formation of cavities in the lesions. Whereas significant roles of cellulolytic enzymes in pathogenesis of tissue degradation were well known as the present studies [1,5,16,32], there were some controversial [23,24,31]. The results of the present study indicate that pectolytic enzymes might be involved in the pathogenesis of the ginseng root rot considering the fact that they were most active on the fourth day after inoculation like Cx was. This view was further supported by many others [4,6,9,11,23,32,35,36] that the macer ation of plant tissues were caused by endo-PG, while others [5,9,22,26,35] were contradictory.

Some authors [8,24,33] reported that PME played a role in the tissue degradation, but this study could not detect the presence of PME as others [3,10] studied. Moreover, transeliminases were regarded as having a role in tissue degradation[7,24,35]. Further experiments on the possible role of transeliminases in ginseng root rot should be extensively done. As many authors studied [1,4,31,33 37] on maceration of plant tissues, many enzymes must be also involved with complex in pathogenesis of ginseng root rot.

The optimum temperatures of cellulolytic and pectolytic enzymes from ginseng roots infected with *C. destructans* were different especially pathogen and/or host.

Cx was inhibited by Hg^{++} at 0.05M like others [12, 13,33]. Some fungicides inhibited the enzyme to some extent at 0.1% of the active ingredient in the present study. This inhibition was probably due to a non-specific salt formation as suggested by and Reese[21]. Although Ca^{++} at 0.1M to 0.2M, Fe^{+++} at 0.01M to 0.02M, and formalin and difolatan at 0.4% to 0.8% inhibited pectolytic enzymes in a great measure, cellulase seemed to be remarkably resistant to inhibition at the same concentrations. In the present study, Fe^{+++} and Al^{+++} were more effective to repress the activities of PG than those of other ions studied. This is in agreement partially with Corden et al's results [14] that Fe^{+++}, Co^{++}, Ni^{++} and Ag^{+} inhibited the PG more than Ca^{++}.

Endo-PG and exo-forms of pectolytic enzymes were inhibited by the ion to considerable extent in this experiment, different from the result that PG was not inhibited by Ca^{++}[15].At 0.2M Ca^{++} and 0.02M Fe^{+++}, the pectolytic enzymes entirely could not be tested owing to coaguration when the enzyme preparations treated were added to the substrates. This coagulation formed to agree with Bateman's result[2] that resistant mechanisms to PG were due to the formation of pectate salt with multivalent cations,particularly Ca^{++}. Although the cellulolytic and pectolytic enzymes produ ced by *C. destructans* were more inhibited by some ions than the fungicides tested, inhibitions of both enzymes must be the subject of further research for the practical control of the ginseng root rot in the field.

It may be concluded that both cellulolytic and pectolytic enzymes produced by *C. destructans* were capable of degradating the two most important constituents of the host cell walls of ginseng root. The cellulolytic enzyme is obviously more associated with the root rot of ginseng than the pectolytic enzymes. The role of the inhibitors on the cellulolytic and pectolytic enzymes could be used to protect ginseng roots from the root rot.

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摘 要

인삼뿌리썩음病菌, *Cylindrocarpon destructans*(Zins.) Scholten을 接種한 人蔘切片으로 부터 얻은 纖維素分解酵素 및 펙틴質分解酵素의 力價는 그 濃度, 時間에 比例하였다. Cellulase (Cx), endo-polygalacturonase (endo-PG), endo-polymethylgalacturonase (endo-PMG), exo-polygalacturonase (exo-PG)와 exo-polymethylgalacturonase(exo-PMG)의 力價는 接種後 4日째 最大였으며 endo-PG와 endo-PMG는 1日 및 2日째는 全혀 檢出되지 않았으나 exo-PG와 exo-PMG는 檢出되었다. 接種後 6日째에는 모든 펙틴質分解酵素는 活性을 完全히 잃었으나 纖維素分解酵素는 높은 力價을 維持하였다. Cx, endo-PG, endo-PMG, exo-PG 및 exo-PMG의 最適 pH는 各各 6.0, 5.5, 8.0, 7.0~7.5, 8.5이였다. Cx, endo-PG, endo-PMG, 및 exo-PG의 最適溫度는 各各 exo-PMG 66°C, 53°C, 41°C, 37°C, 40°C였다. 纖維素分解酵素는 共試한 16個 이온中에서 0.05 M Hg^{++}만이 抑制하였다. 펙틴質分解酵素는 이온에 따라 相異하였으나 0.005M Fe^{+++}과 0.05M Al^{+++}이 가장 顯著히 抑制하였다. 共試한 8個 農藥中에서 어느 것도 0.1% 有效成分으로는 모든 酵素作用을 抑制하지 못했으며 exo-PG만은 모든 農藥의 의하여 相當히 抑制되었다. 그 中에서 Difolatan은 모든 펙틴質分解酵素을 가장 잘 抑制시켰다. 0.2M Ca^{++}과 0.02M Fe^{+++}은 펙틴質分解酵素를 거의 抑制시켰으나 纖維素分解酵素는 같은 濃度에서 各各 30%, 70%에 이르렀다. Formalin은 exo-PG와 endo-PMG를 完全 抑制시켰으나 다른 酵素 특히 Cx는 그렇지 못했다. 0.8% Difolatan은 모든 酵素를 80% 以上 抑制하였으나 Cx는 그 以下였다. *C. destructans*가 分泌하는 纖維素分解酵素 및 펙틴質分解酵素는 人蔘뿌리썩음病과 密接한 關係를 가지고 있으며 이 病을 效果的으로 防除하기 위하여 抑制되어야 하겠다.