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Application of Zymogram for Taxonomy of Phytopathogenic Fungi

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同位酵素를 利用한 病原眞菌類의 分類

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INTRODUCTION

Disease control is one of the most important taskes in agriculture today. Many efforts, such as breeding resistant varieties and application of fungicides, have been made to minimize the damage. However, the effects of them are often diminished within a few years due to appearance and spread of new physiologic races of the pathogens.

Mass transportation of grains and seed exchange between nations have allowed spread of various cultivars rapidly and widely. Consequently, the pathogens become very diversified.

To control the diseases, it is very important to detect and monitor the appearance of new races and pathotypes of fungi. The conventional method for taxonomy of the pathogenic fungi is largely based upon morphological and cultural criteria, and pathogenicity on certain cultivars of plants. However, this method has been experienced not to be satisfactory for detection of genetic variation and determination of genetic relation between isolates. Further, identification of pathotypes so far required lengthy biological testing with resistant and susceptible cultivars. In some species, morphological characters are variable under certain cultural conditions.

To overcome those disadvantages, a new method should be developed. Gel-electrophoresis of protein has received attention as a taxonomic tool(1,4,12). Proteins are functional or structural components, individually shaped for each organisms. With electrophoretic methods, proteins can be separated(2) to form distinct patterns which are characteristic for each individual species or even strains(5,8). Especially, enzymes are products of the genes, enzyme differences reflect genome differences. Comparison of the number of homologous enzymes among groups of the organisms has been used to estimate the amount of genetic similarity or differences between closely related organisms (10). The presence or absence of bands, and the location of bands of certain enzymes can be used for the taxonomy of fungi(5,9,11). *Pyricularia oryzae*(7), *Colletotrichum* spp, and *Ganoderma lucidum* were tested to differentiate species or races of them by electrophoresis.

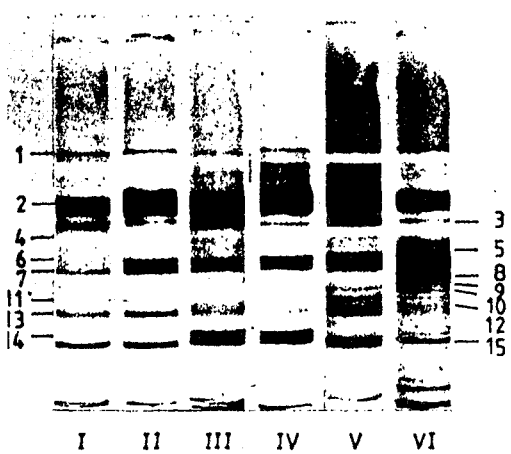
ZYMOGRAMS OF PHYTOPATHOGENIC FUNGI

1. *Pyricularia oryzae* Cav.

Eighty-three isolates of *Pyricularia oryzae* were collected from blasted leaves of rice. Each isolate

as purified by a single spore culture. The isolates ere differentiated into 6 races by the race differenal varieties of rice. The races and number of olates in each race are: KJ-101(8), KJ-201(6), J-301(19), KJ-401(21), KJ-105(19) and KI-315 0). Numbers in parenthesis are the number of olates belonging to the races. At the same time, ıe isolates were divided into 6 zymogram types of sterase (Fig. 1), 4 zymogram types of phosphotase Fig. 2), and 6 zymogram types of catalase (Fig. 3), y electrophoretic patterns of the three enzymes),6). Since there were a few races in one zymogram ype, it could hardly be related with pathogenicity nd one particular zymogram type of the enzyme Table 1). The genetic diversity (percentage simirity) of the fungus was observed by the isozyme atterns of the three enzymes. It was found that the ıngus was genetically heterogenous. The percenıge similarity between isolates was in the range etween 41.1% to 100% (Table 2).

Even the percentage similarity among isolates ithin a race was between 82.1% and 72.2%. This flects the conventional methods of race differenation based only on pathogenicity of an isolate n limited number of race differential varieties ithout consideration of the genetic background of ıe fungus. The isolates were divided into 14 groups y the combination of the zymogram types of the ıree enzymes, so that all isolates within a group



g. 1. Six zymograms of esterase from mycelium of *Pyricularia oryzae* on the 2-30% polyacrylamide gradient gel.

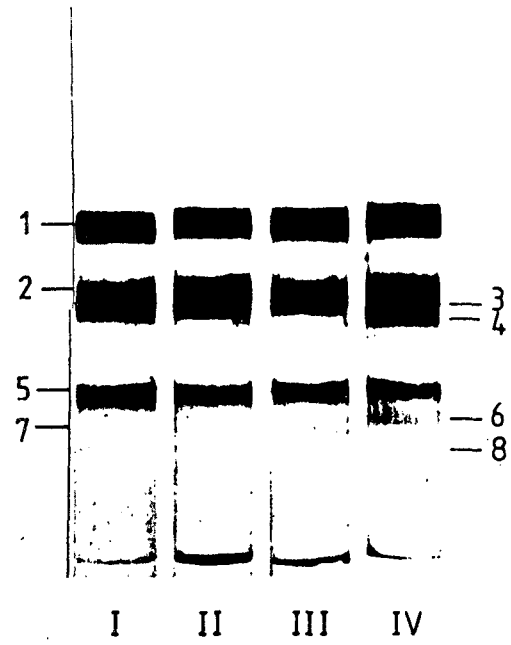


Fig. 2. Four zymograms of phosphotase from mycelium of *Pyricularia oryzae* on 2-30% polyacrylamide gradient gel.

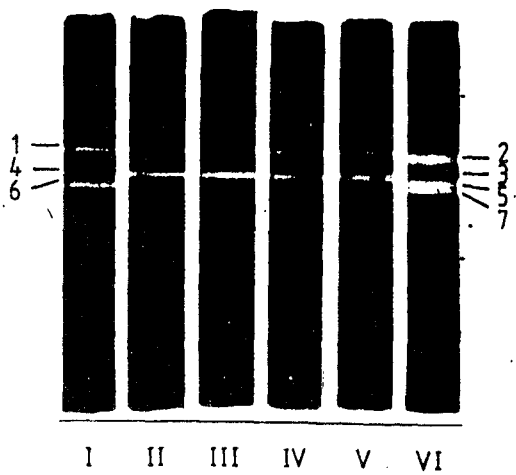


Fig. 3. Six zymograms of catalase from mycelium of *Pyricularia oryzae* on 2-30% polyacrylamide gradient gel.

had similar genetic background as far as the band patterns of the three enzymes were concerned (Table 3).

Even there were few exceptional groups, it was noticed that the isolates within the same group belonged to the same races with similar pathogeni-

Table 1. Races and number of isolates in each zymogram type of *Pyricularia oryzae*

Enzyme	Zymogram type I	II	III	IV	V	VI
estrase	KJ-201[a] 2[b]	KJ-101 5	KJ-101 3	KJ-105 2	KI-315 2	KJ-401 4
	KJ-301 7	KJ-201 2	KJ-201 2	KI-315 4		
	KJ-401 6	KJ-301 7	KJ-301 5			
	KJ-105 5	KJ-401 5	KJ-401 6			
		KI-105 7	KJ-105 5			
			KI-315 4			
phosphotase	KJ-201 2	KJ-101 2	KJ-101 6	KJ-401 4		
	KJ-301 5	KJ-201 2	KJ-201 2	KJ-105 2		
	KJ-401 6	KJ-301 10	KJ-301 4	KI-315 4		
	KJ-105 5	KJ-401 5	KJ-401 6			
		KJ-105 6	KJ-105 6			
		KI-315 4	KI-315 2			
catalase	KJ-301 3	KJ-101 2	KJ-301 4	KJ-101 6	KJ-401 4	KJ-105 2
	KJ-105 1	KJ-201 4	KJ-401 6	KJ-201 2		KI-315 4
		KJ-301 6	KI-315 2	KJ-301 6		
		KJ-401 7		KJ-401 4		
		KJ-105 11		KJ-105 5		
		KI-315 4				

[a] race

[b] numbers of isolates in race.

Table 2. Percentage similarity among groups of *Pyricularia oryzae*

Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	–	80.5	75.8	86.9	75.8	81.9	70.8	86.1	75.0	70.0	70.0	45.5	63.0	41.1
2	–	–	84.1	84.1	73.0	77.4	66.3	83.3	72.2	87.8	65.5	57.5	80.8	58.9
3	–	–	–	88.9	77.8	82.2	71.1	79.3	68.2	72.7	88.9	52.1	69.7	47.8
4	–	–	–	–	88.9	93.3	82.2	90.5	79.3	72.7	72.7	52.1	69.7	47.8
5	–	–	–	–	–	82.2	93.3	79.3	60.5	72.7	83.8	60.5	58.6	58.9
6	–	–	–	–	–	–	88.9	83.8	72.7	79.4	79.3	58.3	76.4	54.4
7	–	–	–	–	–	–	–	72.7	83.8	79.3	90.5	67.1	65.3	65.5
8.	–	–	–	–	–	–	–	–	88.9	82.2	82.2	56.9	73.9	63.0
9.	–	–	–	–	–	–	–	–	–	71.1	93.3	65.2	62.8	63.0
10	–	–	–	–	–	–	–	–	–	–	77.8	71.9	91.7	69.7
11	–	–	–	–	–	–	–	–	–	–	–	71.9	69.4	69.7
12	–	–	–	–	–	–	–	–	–	–	–	–	72.5	79.2
13	–	–	–	–	–	–	–	–	–	–	–	–	–	62.8
14	–	–	–	–	–	–	–	–	–	–	–	–	–	–

city. The results of the preliminary tests showed affected by cultural conditions, regardless the age from 5 to 45 days of culture, carbon sources such as from 5 to 45 days of culture, carbon sources such as glucose, fructose, sucrose, starch, cellulose and glyceline, or nitrogen sources, such as yeast extract and peptone. The mycelial growth in inorganic nitrogen sources which were potassium nitrate and ammonium sulfate was too poor to be used for enzyme extraction. The most important thing was separation method. We tested 4 different gels, which were 7% homogenous polyacrylamide gel, starch gel, isoelectric focusing gel with 1% Servaylet pH 2-14, and gradient polyacrylamide gel (2-30%). The 2-30% gradient polyacrylamide gel produced more distinct and greater numbers of bands than

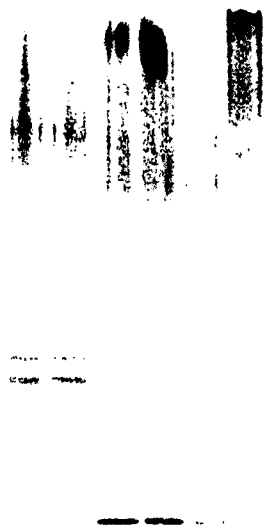
Table 3. Groups of isolates by combination of zymogram types of three enzymes of *Pyricularia oryzae*

Group	Zymogram type			Race (number)
	estrase	phosphotase	catalase	
1	I	I	II	KJ-201 (2), KJ-301 (3), KJ-401 (6), KJ-105 (5).
2	I	II	III	KJ-301 (4).
3	II	II	I	KJ-301 (3), KJ-105 (1).
4	II	II	II	KJ-101 (2), KJ-201 (2), KJ-301 (1) KJ-401 (1), KJ-105 (1).
5	II	II	IV	KJ-401 (4).
6	II	III	II	KJ-105 (5).
7	II	III	IV	KJ-101 (3), KJ-301 (1).
8	III	II	II	KJ-315 (4).
9	III	II	IV	KJ-301 (2), KJ-105 (4).
10	III	II	III	KJ-401 (6).
11	III	III	IV	KJ-101 (3), KJ-201 (2), KJ-301 (3), KJ-105 (1).
12	IV	IV	VI	KJ-105 (2), KI-315 (4).
13	V	III	III	KI-315 (2).
14	VI	IV	V	KJ-401 (4).

other gels.

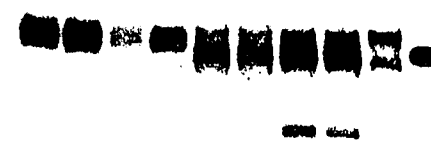
2. *Colletotrichum* spp.

Anthracnose, caused by *Colletotrichum* spp., would damage on many kinds of horticultural crops. *C. gloeosporioides* has occurred on the fruits of red papper. There are two strains in *C. gloeosporioides* which are R-strain and G-strain. The R-strain can infect and develope symptom only on ripen stage, red, of the fruit. While the G-strain can infect all



1 2 3 4 5 6 7 8 9 10

Fig. 4. Esterase isozyme patterns from mycelium of *Colletotrichum* spp. on 10-25% polyacrylamide gradient gel. 1, 2, 3, 4,: G-strains of *C. gloeosporioides*, 5, 6, 7, 8,: R-strains of *C. gloeosporioides*, 9: *C. cingulata*, and 10: *C. dematium*.



1 2 3 4 5 6 7 8 9 10

Fig. 5. Phosphotase isozyme patterns from mycelium of *Colletotrichum* spp. on 10-25% polyacrylamide gradient gel. 1, 2, 3, 4,: G-strains of *C. gloeosporioides*, 5, 6, 7, 8,: R-strains of *C. gloeosporioides*, 9: *C. cingulata*, and 10: *C. dematium*.

the stages of the fruits, green through red. By the zymograms of esterase (Fig. 4) and phosphotase (Fig. 5), the two strains were easily differentiated. Also, the patterns of the enzymes of *C. cingulata* and *C. dematium* had distinct shape. The species of *Collectotrichum* were differentiated by the zymograms.

3. *Trichoderma* spp.

Trichoderma spp. were considered as a beneficial microorganisms in soil. For their strong antagonistic activities to other soil microorganisms, it has been studied for biological control agent. On the other hand, the fungus used to occur on mushroom bed. Mushroom growers have been suffered much damage from the fungus. It is very difficult to differentiate the species of *Trichoderma*, because the taxonomy of the fungus is based on the color and zonation of colony, and shape of conidiophore. Since those characters are affected by cultural conditions, it is very difficult to identify the species, *T. viride*, *T. harzianum*, *T. koningi*, *T. polyspor* *T. pseudokiningi* and *T. hamatum*. The isozym patterns of esterase showed distinct patterns of eac species. (Fig. 6). By the zymograms of the enzym the species of *Trichoderma* can be easily identifie

4. *Ganoderma lucidum*

Ganoderma lucidum is one of the medicina mushroom. Due to high demand of it, many mu shroom growers have raised the mushroom in larg scale. There are 16 strains of the mushroom ir Korea. Since most of the strains were importec from foreign countries without consideration o genetic background, their genetic background is no clear. It might be possible that some of them were originated from a same source, even their strain numbers were different. However, the characteri stics of the fruiting body are hardly used for strain identification. Because, the shapes of the fruiting bodys are so alike among them and variable unde cultural conditions. The isozyme patterns of ester ase showed the difference or similarity betwee strains. (Fig. 7). the patterns can be used to diff erentiate the strains of the *G. lucidum*.

The electrophoresis is very useful method tc

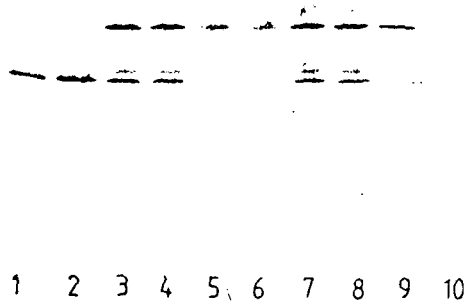


Fig. 6. Estrase isozyme patterns of *Trichoderma* spp. on 10-25% polyacrylamide gradient gel. 1, 2: *T. viride*, 3, 4: *T. harzianum*, 5, 6: *T. koningi*, 7, 8: *T. polysporum*, 9: *T. pseudokoningi*, 10: *T. hamatum*.

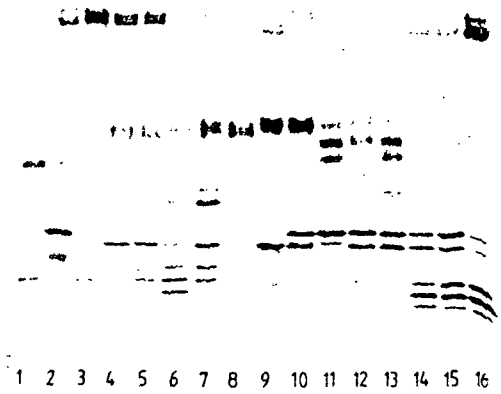


Fig. 7. Esterase patterns from cap of *Ganoderma lucidum*. on 10-25% polyacrylamide gradient gel. 1 to 16: Strain number of the fungus.

ifferentiate the closely related fungi. This method hould be considered as a supplementary method or conventional taxonomy.

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