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Preliminary Studies of Isozyme Patterns of Isolates of *Colletotrichum gloeosporioides* from Host Plants in Malaysia

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ABSTRAK

Variasi isozim pada 25 isolat Colletotrichum gloeosporioides *daripada 12 perumah tumbuhan di Malaysia telah dikaji menggunakan polyacrylamide gel elektroforesis. Perumah-perumah tersebut termasuk tujuh jenis daripada legum, dan hanya satu jenis daripada citrus, rumput, koko, lada hitam dan cili. Satu hingga lima elektromorph telah dihasilkan oleh setiap enzim pada lima sistem enzim yang dikaji. Ada juga isolat yang tidak menghasilkan sebarang jalur. Dua jalur dan jalur multipel dapat diperhatikan pada esterase, MDH, GLDH dan LDH. Hanya isolat lada hitam menghasilkan satu jalur pada GDH. Terdapatnya variasi pada corak jalur esterase dan MDH pada isolat-isolat daripada perumah yang berlainan. Kajian isozim berkemungkinan dapat digunakan untuk mengenalpasti* C. gloeosporioides *daripada perumah yang berlainan apabila lebih banyak sampel dikaji di masa yang akan datang.*

ABSTRACT

Isozyme variation of 25 isolates of Colletotrichum gloeosporioides *from 12 different Malaysian host plants were studied using polyacrylamide gel electrophoresis. The hosts included seven species of legumes and one species each of citrus, grass, cocoa, pepper and chilli. One to five electromorphs per enzyme were produced for the five enzyme systems typed. Some isolates did not produce any bands for the enzymes studied. Double to multiple bands were observed with esterases, MDH, GLDH and LDH. Only one pepper isolate produced a single band for GDH. Some variations among the isolates of different host plants were observed with the isozyme patterns of esterases and MDH. Isozyme tests may prove to be a useful tool in the identification of* C. gloeosporioides *of different host plants when more samples are tested in future.*

INTRODUCTION

The taxonomy of *Colletotrichum* is based mainly on conidial morphology and size (Arx, 1957; Sutton, 1980). Several host specific forms exist among the species of *Colletotrichum* which make delimitation of the species difficult. Anthracnose disease caused by *Colletotrichum gloeosporioides* is widespread and damaging to many varieties of hosts. *C. gloeosporioides* is characterised by straight conidia with rounded or at times pointed ends, ranging 12-19 mm long (Arx, 1957) and 5-35 mm long (Davies *et al.*, 1992). The conidia are normally produced on conidionematous phialides within an acervulus. Some isolates of *C. gloeosporioides* exhibit presence of setae on the acervulus. Conidia can also be produced directly from the hyphae. Other straight-spored producing species of *Colletotrichum* are *C. musae*, and *C. crassipes*. Conidial size and shape alone are inadequate to categorize *C. gloeosporioides*. Electrophoretic studies have been used over the past few decades to analyse the variation among the physiological races (Gill and Powell, 1968) and the virulence in natural populations (Lenne and Burdon, 1990) of various fungi. The aim of this study was to investigate whether there is any isozyme difference existing among isolates of *C. gloeosporioides* isolated from some Malaysian host plants.

MATERIALS AND METHODS

Twenty five isolates of *C. gloeosporioides* were isolated from 12 host plants (Table 1). Isolates studied included two from *Citrus reticulata* (mandarin orange), three from *Piper nigrum* (pepper), two from *Capsicum frutescens* (chilli pepper), three from *Imperata cylindrica* (a grass), one from *Vigna radiata* (mung bean), two from *Theobroma cacao* (cocoa), four from *Centrosema pubescens* (a legume), one from *Pueraria phaseoloides* (a legume), one from *Calopogonium mucunoides* (leguminous cover crop), two from *Phaseolus vulgaris* (French bean), one from *Clitoria ternatea* (butterfly pea) and three from *Psophocarpus tetragonolobus* (winged bean). All monospored cultures were maintained on Potato Dextrose Agar (PDA).

Polyacrylamide Gel Electrophoresis

Discs 3 mm in diameter from each of the fungal isolates were transferred into McCartney bottles containing Malt extract broth. The cultures were then incubated for 10 days in the dark to enhance mycelial growth versus spore production. The mycelia were then harvested by washing five times with sterile distilled water, filtering through muslin cloth, blotting dry and freezing at about -20°C. The mycelia were then ground with a drop from a pasteur pipette of extraction buffer pH 8.0 (0.05 M Trizma base, 0.1 mM b-Nicotinamide Adenine Dinucleotide (NAD), 0.01M Glycyl glycine, 0.01M CaCl2 and 0.01 mM b-Nicotinamide Adenine Dinucleotode Phosphate (NADP) in 1 l distilled water. Small strips of filter paper (1 x 2 mm) Whatman no. 17 were soaked in each of the ground mycelial fluids, then placed on the base of polyacrylamide gel pH 7.2, 2.0 mm thick, 19 x 19 cm L/W. A marker, Bromophenol Blue, was used on either side of the gel. The gel was allowed to run on horizontal gel electrophoretic system with the Tris-citric acid electrode buffer pH 6.9 (0.135 M Trisma base and 0.040 M citric acid) at a constant current at 50 mA until the buffer front reached

TABLE 1

Isolate number from host plants of *Colletotrichum gloeosporioides*

Isolate number	Host	Family
Imp. 18 (ii)	*Imperata cylindrica*	Gramineae
Imp. 18 (i)	*Imperata cylindrica*	Gramineae
Imp. C 012.2	*Imperata cylindrica*	Gramineae
CM	*Citrus reticulata*	Rutaceae
CM002	*Citrus reticulata*	Rutaceae
Pip B 001	*Piper nigrum*	Piperaceae
Pip B 004	*Piper nigrum*	Piperaceae
Pip B oo5	*Piper nigrum*	Piperaceae
Cp 011	*Theobroma cacao*	Sterculiaceae
Cp 013	*Theobroma cacao*	Sterculiaceae
Chi 003	*Capsicum frutescens*	Solanaceae
Chi 005	*Capsicum frutescens*	Solanaceae
Peu B	*Pueraria phaseoloides*	Leguminosae
32 Khst 1.3	*Vigna radiata*	Leguminosae
CI 003	*Clitoria ternatea*	Leguminosae
Cent st	*Centrosema pubescens*	Leguminosae
27 Cent B 1.3	*Centrosema pubescens*	Leguminosae
2 Kb f 1.5	*Psophocarpus tetragonolobus*	Leguminosae
4 Kb L1.3	*Psophocarpus tetragonolobus*	Leguminosae
Pt 004	*Psophocarpus tetragonolobus*	Leguminosae
PV 001	*Phaseolus vulgaris*	Leguminosae
CAF	*Calopogonium mucunoides*	Leguminosae

about 11 cm. The gels were then stained with the respective stains (Harris and Hopkinson, 1976) for each of the isozymes tested. The isozymes tested were esterase (Est), lactate dehydrogenase (LDH), glucose dehydrogenase (GDH), glutamate dehydrogenase (GLDH) and malate dehydrogenase (MDH).

RESULTS AND DISCUSSION

The isozyme patterns or the electromorphs of the 5 enzyme systems found in 25 isolates of *C. gloeosporioides* are given in *Fig. 1* and the isozyme phenotypes of isolates from 12 host species are given in Table 2. The positions of the bands in *Fig. 1* are based on the migration distance on the gel and the Rf values calculated. One to five isozyme patterns occurred per enzyme with a total of twelve for the five enzymes (Fig. 1). For esterase and malate dehydrogenase both single and multi-banded patterns were found among the isolates. Glucose dehydrogenase showed a single-banded pattern, glutamate dehydrogenase produced a double banded pattern while lactate dehydrogenase produced a three-banded pattern. Single banded phenotypes wherever produced may be controlled by a single gene. Isozyme data are interpreted conservatively, considering only the banding phenotypes where each banding pattern per enzyme and isolate was determined as a single electromorph.

GLDH showed the two-banded isozyme profile for the *C. gloeosporioides* of the winged bean only and no profile for any of the other 24 isolates studied. GDH showed the one-banded isozyme profile for a pepper isolate only. For LDH, 13 out of 25 isolates produced a three-banded isozyme profile. The 13 isolates were from unrelated species of host plants. The banding pattern can be said to be homogenous and therefore LDH was not a good marker. Dehydrogenases are difficult to stain and resolve.

Fifty percent of the isolates tested gave four types of banding patterns when stained for MDH. The band pattern 1 with 4 bands was shown by seven isolates namely, two of pepper, two from *Centrosema*, two from French bean and one from winged bean. The pattern 2 with 3 bands for MDH was shown only by the three isolates of grass. The single-band pattern 3 was produced

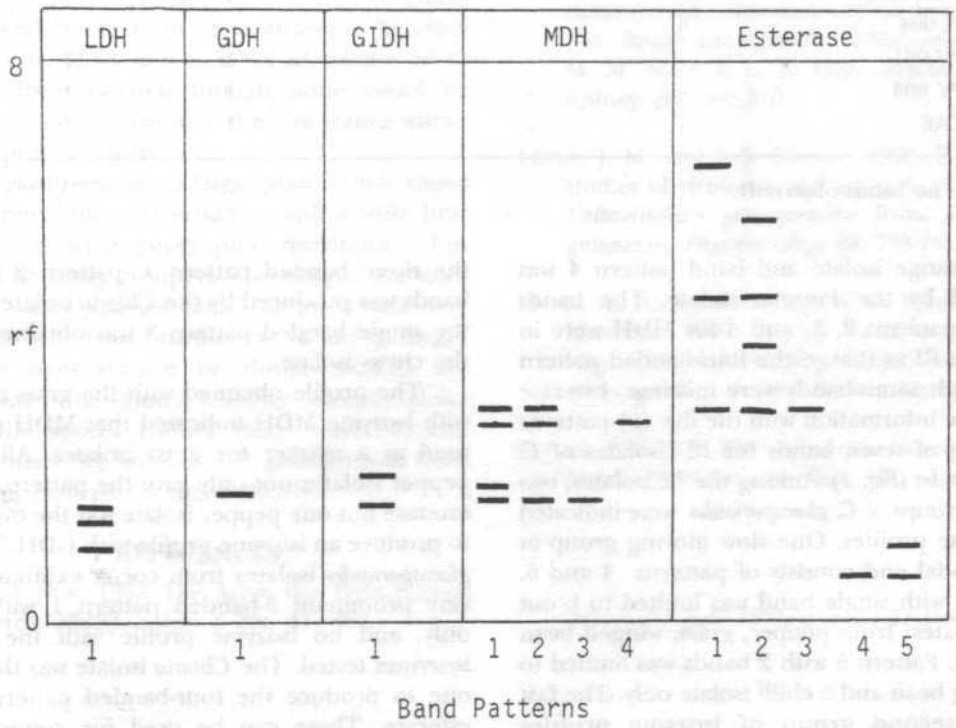

Fig. 1: Isozyme patterns for five enzyme systems from Colletotrichum gloeosporioides *from host plants in Malaysia*

TABLE 2
Isozyme phenotypes of isolates of *Colletotrichum gloeosporioides*

Isolate number	EST	GDH	GLDH	LDH	MDH
Imp. 18 (ii)	-	-	-	-	2
Imp. 18 (i)	-	-	-	-	2
Imp. C 012.2	4	-	-	-	2
CM	-	-	-	1	3
CM002	3	-	-	-	-
Pip B 001	4	-	-	-	-
Pip B 004	4	1	-	1	1
Pip B 005	4	-	-	-	1
Cp 011	1	-	-	-	-
Cp 013	1	-	-	-	-
Chi 003	4	-	-	-	-
Chi 005	5	-	-	-	-
Peu B	-	-	-	1	4
32 Khst 1.3	5	-	-	-	-
C1 003	2	-	-	-	-
Cent st	-	-	-	1	1
25 Cent st 1.3	-	-	-	1	-
Cent st a	-	-	-	1	-
27 Cent B 1.3	-	-	-	1	1
2 Kb f 1.5	-	-	-	1	-
4 Kb L1.3	-	-	1	1	1
Pt 004	4	-	-	-	-
PV 001	-	-	-	1	1
PV 003	-	-	-	1	1
CAF	-	-	-	1	-

(- no bands observed)

by the orange isolate and band pattern 4 was produced by the *Pueraria* isolate. The bands seen in patterns 2, 3 and 4 for MDH were in the same Rf as that of the four-banded pattern 1, although some bands were missing. Esterase gave more information with the five (5) patterns consisting of seven bands for 12 isolates of *C. gloeosporioides (Fig. 1)*. Among the 12 isolates, two distinct groups of *C. gloeosporioides* were indicated by esterase profiles. One slow moving group at the cathodal end consists of patterns 4 and 5. Pattern 4 with single band was limited to 6 out of 12 isolates from pepper, grass, winged bean and chilli. Pattern 5 with 2 bands was limited to the mung bean and a chilli isolate only. The fast moving second group of isozyme profiles consisted of patterns 1, 2 and 3 and was limited to only four isolates. The cocoa isolates produced the three banded pattern 1; pattern 2 with 4 bands was produced by the *Clitoria* isolate; while the single banded pattern 3 was obtained with the citrus isolate.

The profile obtained with the grass isolates with isozyme MDH indicated that MDH can be used as a marker for grass isolates. All three pepper isolates not only gave the pattern 4 with esterase but one pepper isolate was the only one to produce an isozyme profile with GDH. The *C. gloeosporioides* isolates from cocoa exhibited the very prominant 3-banded pattern 1 with EST only, and no isozyme profile with the other isozymes tested. The *Clitoria* isolate was the only one to produce the four-banded pattern with esterase. These can be used for comparative purposes. The citrus isolate CM002 was the only one to produce the one-banded, Rf 0.318, pattern

3, with esterase while the other isolate CM was the only one to produce the single banded pattern 3 with MDH at Rf 0.173.

Considerable variations in phenotype exist among the isolates of *C. gloeosporioides* from different host plants. The *C. gloeosporioides* from different hosts exhibited some variation in the isozyme patterns, especially when typed for esterase and malate dehydrogenase. Some specific patterns were also seen especially for MDH for the grass isolates, esterase and GDH for pepper, esterase for cocoa isolates, esterase for *Clitoria* and esterase and MDH for citrus isolates.

In studies on many isolates of the anthracnose pathogen *C. gloeosporioides of Stylosanthes guianensis*, the fungus was reported to belong to two groups on the basis of the conidial morphology, disease symptoms, host range and virulence on certain cultivars (Irwin *et al.*, 1984), on their double stranded RNA (Dale *et al.*, 1988) and by electrophoretic karyotype (Masel *et al.*, 1990). Lenne and Burdon (1990) showed considerable phenotypic variation within five natural populations of *C. gloeosporioides* collected from *S. guianensis* and correlated sexual reproduction to the variation that exists among the population. Hodson *et al.* (1993) found considerable variation in restriction banding pattern in rDNA and mtDNA of species of *C. gloeosporioides* isolates, though none could be distinguished in relation to the host source within geographic localities.

C. gloeosporioides is a large group which shows great morphological variation with a wide host range and wide geographic distribution but cannot be easily grouped. The sample size used in this study was too small to make any firm conclusions. Thus, further work using larger sample sizes should be done. Studies are underway to determine whether esterase alone from the spores (rather than mycelia) can differentiate the isolates of *C. gloeosporioides* from different host plants more clearly.

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