Isozyme Analysis and Aminopeptidase Activities Within the Genus *Peronosclerospora*

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ABSTRACT

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Electrophoresis and aminopeptidase assays were used to study the taxonomy within the genus *Peronosclerospora*. The banding patterns of 26 enzymes were determined by horizontal starch gel electrophoresis, and coefficients of similarity were calculated among isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis*. Isolates of *P. sorghi* from Thailand were not closely related to any other isolate in this study. Isolates of *P. sacchari* and *P. philippinensis* exhibited identical phenotypes for 22 enzymes and probably represent a single species. *P. maydis* shared phenotypes with *P. sacchari* and the Thailand isolates but also displayed unique banding patterns in certain enzyme systems. Electrophoresis readily separated isolates of *Peronosclerospora* from those of *Bremia lactucae* and *Peronospora tabacina*. Aminopeptidase assays could not differentiate isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis* due to large amounts of intraspecific variation.

Additional keywords: corn diseases, downy mildows, fungal genetics, fungal taxonomy, Oomycetes, sugarcane diseases.

Members of the genus *Peronosclerospora* cause several important downy mildew diseases of maize, sugarcane, and sorghum in the tropical and subtropical regions of the world. These diseases are often the limiting factor in crop production in developing countries and preclude the planting of otherwise preferred varieties. *Peronosclerospora sorghi* (Weston & Uppal) C. G. Shaw, the causal agent of sorghum downy mildew, is the only species present in the Western Hemisphere; it primarily affects sorghum, although maize is also susceptible. Disease incidence in Texas has exceeded 30% and can be much higher in regions favorable to disease development (10). *Peronosclerospora philippinensis* (Weston) C. G. Shaw, the causal agent of Philippine downy mildew of maize, and *P. sacchari* (T. Miyake) Shirai & Hara, the causal organism of sugarcane downy mildew, have produced field losses of 40–60% among susceptible maize varieties in the Philippines (9) and 70% in Taiwan (23). The development of resistant varieties has reduced the incidence and severity of these diseases in Asia, but American varieties remain highly susceptible (21). *P. heteropogoni* Siradhana, Dange, Rathore & Singh, present in northern India, and *P. maydis* (Racib.) C. G. Shaw, in Indonesia, can also be very serious. Rapid and accurate means of identification of these organisms are necessary to prevent their introduction and establishment in new regions.

Identification of *Peronosclerospora* species is difficult due to the minor morphological differences that separate the species. The described species are differentiated by only slight variations in the size and shape of the conidia and conidiophores; host range, symptom expression, and oospore production are also used to distinguish among species (26,27). Morphological criteria can be highly variable; however, Pupipat (19) was able to segregate 32 isolates of *P. sorghi* on maize into 10 categories based on conidial shape. Conidial dimensions are highly dependent on host species (8), cultivar (15), and the environmental conditions at the time of sporulation (15).

Electrophoresis of individual enzymes, as detected by specific activity stains, is becoming a widely used technique in mycology and plant pathology. The subject has been recently reviewed (16). In an initial study, electrophoresis was used to differentiate among three *Peronosclerospora* species (4). The banding patterns of 12 enzymes were determined among six, two, and two isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis*, respectively. This experiment revealed a close biochemical relationship between *P. sacchari* and *P. philippinensis*, but a distant association between the isolate of *P. sorghi* from Thailand and those collected in Texas, India, and Brazil. The limited number of available isolates reduced the amount of interpretable information since the amount of true intraspecific variation could not be accurately estimated.

Aminopeptidase assays can also be used to study the taxonomy of a group of organisms through their biochemistry. These assays are based on the amount of fluorescent β-naphthylamine that is enzymatically released from nonfluorescent β-naphthylamide derivatives of amino acids. A linear relationship has been found between the concentration of β-naphthylamine and the intensity of the fluorescence; this relationship allows quantitative comparisons of aminopeptidase activities among isolates. This technique has been used in the identification of medically important bacteria (25) and has also been tested with certain plant pathogens. Huber et al (12) were able to differentiate four species of *Phytophthora* by their differing aminopeptidase profiles. The procedure has also been used to identify races of *Bipolaris maydis* (24).

The objective of this paper was to study further the taxonomic and genetic relationships within the genus *Peronosclerospora*. Electrophoretic studies were expanded from the previous work (4). Additional isolates of the three species, including isolates of *P. sorghi* from Honduras, Argentina, and Ethiopia were incorporated so that measures of intra- and interspecific variation would be more meaningful. Isolates of a fourth species, *P. maydis*, were also included in this study. Intergeneric comparisons were made between *Peronosclerospora*, *Bremia lactucae* Regal, and *Peronospora tabacina* Adam, causal agents of lettuce downy mildew and blue mold of tobacco, respectively. Additional enzyme systems were examined. A preliminary account of this work has been presented (17). Isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis* were also assayed for aminopeptidase activities to provide additional information about the taxonomic relationships of these organisms.

MATERIALS AND METHODS

Acquisition and maintenance of cultures. Isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis* were obtained from field collections of diseased hosts; the location and date of collection and the names of the collectors are listed in Table 1. Field identifications were verified by morphological examination of the isolates (2), host range studies (3), and symptom expression (7). Isolates of *P. sacchari*, *P. philippinensis*, and those of *P. sorghi* from Thailand were established from conidia collected from systemically infected maize and sugarcane. Cultures of *P. sorghi* from Texas, Honduras, Argentina, Brazil, India, and Ethiopia were derived from oospores formed in systemically infected sorghum. All isolates of *P. sorghi*, with the exception of those from Thailand, were of the maize-sorghum strain (i.e., able to infect and colonize both maize and sorghum); the Thailand isolates would grow only on maize. The cultures were maintained by monthly conidial inoculations of maize (cultivar Pioneer 3369A) seedlings.

TABLE 1. Isolate numbers, source, and dates of collection of *Peronosclerospora* species

Species	Isolate no.	Location and year of collection	Source
P. sorghi	01	Port Lavaca, Texas, 1972	R. A. Frederiksen
	02	Port Lavaca, Texas, 1981	J. Craig
	11	Pak Chang, Thailand, 1975	R. Renfro
	12	Pak Chang, Thailand, 1985	C. DeLeon
	21	Soledad, Honduras, 1983	G. Wall
	22	Comayagua, Honduras, 1984	G. Wall
	31	Mysore, India, 1980	S. S. Bhat
	32	Mysore, India, 1982	M. R. Bonde
	33	Dharwar, India, 1983	L. Mughogho
	34	Patancheru, India, 1983	L. Mughogho
	41	Jaboticabal, Brazil, 1982	N. G. Fernandes
	44	Brazil, 1983	L. Giorda
	71	Cordoba Province, Argentina, 1983	L. Giorda
	72	La Pampa Province, Argentina, 1983	L. Giorda
	73	Argentina, 1982	E. Teyssandier
	83	Samaru, Nigeria, 1984	L. E. Claflin
P. sacchari	01	Tainan, Taiwan, 1975	S. C. Chang
	02	Tainan, Taiwan, 1983	S. C. Chang
	03	Tainan, Taiwan, 1984	S. C. Chang
	04	Taiwan, 1985	S. C. Chang
	05	Taiwan, 1985	S. C. Chang
	06	Taiwan, 1985	S. C. Chang
	21	Papua, New Guinea	...
	23	Papua, New Guinea	...
P. philippinensis	01	Los Banos, Philippines, 1975	O. Exconde
	02	Los Banos, Philippines, 1979	M. R. Bonde
	04	La Granja Exp. Stn., Philippines, 1984	F. R. Husmillo
	06	La Granja Exp. Stn., Philippines, 1984	F. R. Husmillo
	08	La Granja Exp. Stn., Philippines, 1984	F. R. Husmillo
	11	Luzon Exp. Stn., Pampanga, 1984	J. M. Bonman
	12	Luzon Exp. Stn., Pampanga, 1984	J. M. Bonman
	14	Luzon Exp. Stn., Pampanga, 1984	J. M. Bonman
P. maydis	01	West Java, Indonesia	H. Vermeulen
	02	East Java, Indonesia	H. Vermeulen

Isolates of *P. sacchari* and *P. philippinensis* were also maintained on sugarcane for long-term storage. All manipulations with the living fungus and infected plants were conducted within the containment facility of the USDA-ARS Foreign Disease-Weed Science Research Unit at Fort Detrick in Frederick, MD.

Production and treatment of conidia for electrophoresis. Conidia were produced and collected as previously described (4). A conidial suspension of each isolate containing 5×10^3–5×10^4 conidia per milliliter was sprayed onto 60 maize plants in the two-to three-leaf stage using an atomizer with 3,500 kg/m^2 (5 lb/m^2) airline pressure. After inoculation, all plants were incubated overnight in dew chambers at 20 C and then placed in the greenhouse for 21–30 days. Sporulation was induced by exposing the systemically infected plants to supplemental light (1,000 W Sylvania Metalarc high-intensity lamps) for 16 hr and then placing them in dark dew chambers at 20 C for 5–7.5 hr. Conidia were collected by washing the leaves with a fine spray of cold (5 C) distilled water delivered by an atomizer at about 3,500 kg/m^2 (5 lb/in^2) airline pressure. The spore suspensions were immediately filtered through a 150-μm (100 mesh) screen and centrifuged at 700 *g* for 5 min. After centrifugation, most of the water was removed by pipette, leaving about 2 ml in which the pellet was resuspended. The spore suspension was transferred to a 2-ml Nunc round-bottomed cryotube (Thomas Scientific Co., Swedesboro, NJ) and stored in liquid nitrogen until used. Each sample contained approximately $2–5 \times 10^6$ conidia.

Frozen samples were removed from containment and taken to the electrophoresis laboratory in liquid nitrogen. Each frozen sample was crushed with a cold 8-mm-diameter glass rod, the end of which had been melted to form a bulb that fit snugly into the Nunc tube. About 40–60% of the conidia were ruptured by this treatment. The crushed samples were transferred to 12- × 75-mm

TABLE 2. List of enzymes with detectable activity, enzyme abbreviations, enzyme commission numbers, and buffer systems

Enzyme	Abbrev.	Enzyme commission number	Buffer system
Acid phosphatase	ACP	3.1.3.2	4[a]
Aconitase	AC	4.2.1.3	4
Adenylate kinase	AK	2.7.4.3	4
Alpha-glycerophosphate dehydrogenase	AGP	3.2.1.20	4
Aspartate aminotransferase	AAT	2.6.1.1	C
Diaphorase	DIA	1.6.4.3	R[b]
Esterase	EST	3.1.1.1	R
Fructose diphosphatase	FDP	3.1.3.11	4
Glucose phosphate isomerase	GPI	5.3.1.9	C[c]
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	R
Glucokinase	GK	2.7.1.2	4
β-Glucosidase	β-GLU	3.2.1.21	R
Glutamate dehydrogenase	GDH	1.4.1.3	R
Glutamic pyruvic transaminase	GPT	2.6.1.2	R
Glutathione reductase	GR	1.6.4.2	4
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	1.2.1.12	C
Isocitrate dehydrogenase	IDH	1.1.1.42	R
Malate dehydrogenase	MDH	1.1.1.37	C
Mannitol dehydrogenase	MADH	1.1.1.67	4
Mannose phosphate isomerase	MPI	5.3.1.8	R
Peptidase with glycyl-leucine	PEP-GL	3.4.11 or	R
Peptidase with leucyl-leucyl-leucine	PEP-LLL	3.4.13	R
Peptidase with phenyl-alanyl-proline	PEP-PAP		R
Phosphogluconate dehydrogenase	PGD	1.1.44	C
Superoxide dismutase	SOD	1.11.1.1	R
Triose phosphate isomerase	TPI	5.3.1.1	R
Xanthine dehydrogenase	XDH	1.2.1.37	R

[a] Gel buffer = 0.005 M Tris + 0.003 M citric acid, pH 6.7. Electrode buffer = 0.068 M Tris + 0.037 M citric acid, pH 6.3 (B. May, *personal communication*). Electrophoresis run at ≤250 V, 75 mA for 2.5 hr.

[b] Electrode and gel buffers as described by Ridgway et al (20). Electrophoresis run at ≤250 V, 75 mA for 2.5 hr.

[c] Electrode buffer as described by Clayton and Tretiak (6), diluted 1:10 for gel buffer. Electrophoresis run at ≤200 V, 90 mA for 2.5 hr.

test tubes and brought to 0.2–0.5 ml with sample buffer (0.05 M Tris-Cl, pH 7.1). Solid debris were removed by centrifugation.

Gel electrophoresis. Horizontal starch gel electrophoresis was performed as described by Micales et al (16). The enzymes that were assayed are listed in Table 2. Simple matching coefficients (SSM) were determined for each pair of isolates as described by Sokal and Michener (22) by the formula:

$$SSM = m/(m + u),$$

where m = the number of pairs of protein bands found in common between the two samples, and u = the total number of bands unique to each sample.

Aminopeptidase assay. The assay for aminopeptidases was conducted as described by Huber et al (12). Aminopeptidase profiles were determined for the Thailand isolate (#11) and isolates of *P. sorghi* (#72, 32), *P. sacchari* (#01, 02, 03), and *P. philippinensis* (#01, 02, 04, 08, 11, 12, 14). The assay was conducted by adding 0.1 ml of a conidial suspension (5×10^5 spores/ml in 0.05 M Tris-Cl, pH 8.0) to 1.8 ml of 2.5×10^{-5} M β-naphthylamide substrate in 0.05 M Tris-Cl, pH 8.0. The assays were incubated at room temperature (approximately 25 C) for 5 and 24 hr. Fluorescence of the enzymatically released β-naphthylamine was measured at 540 nm with an Aminco Fluoro-Colorimeter (American Instrument Co., Silver Spring, MD). Each assay was performed in duplicate. Percent hydrolysis, or relative fluorescence, was calculated by the formula:

$$\% \text{ hydrolysis} = \frac{{}^{F}\beta\text{-naphthylamide substrate} - {}^{F}\beta\text{-naphthylamine control}}{{}^{F}\beta\text{-naphthylamine control} - {}^{F}\text{buffer}} \times 100$$

where F = fluorescence.

The beta-naphthylamine substrates used in this study were: L-alanyl (ALA), L-arginyl (ARG), benzoylarginyl (BARG), L-alpha-aspartyl (ASP), L-cystinyl (CYS), L-gamma-glutamyl (GLU), L-glycyl (GLY), L-histidyl (HIS), L-hydroxyprolyl (HPRO), L-leucyl (LEU), L-isoleucyl (ILEU), L-lysyl (LYS), L-methionyl (MET), 4-methoxyalanyl (MALA), 4-methoxyleucyl (MLEU), L-phenylalanyl (PHE), L-prolyl (PRO), L-pyrolidonyl (PYR), L-seryl (SER), L-tryptophyl (TRY), L-tyrosyl (TYR), and L-valyl (VAL). Significant differences in hydrolysis levels were determined by Duncan's multiple range test.

RESULTS

Electrophoresis. Isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis* were tested for 30 different enzyme activities in three different buffers. Poor resolution of electrophoretic bands or low levels of activity were obtained for the enzymes: lactate dehydrogenase, fructose bisphosphate aldolase, fumarase, and leucine aminopeptidase. The remaining enzymes are listed in Table 2 with the buffer system that gave optimal resolution.

Most isolates of *P. sorghi* sporulated poorly; it was not possible to produce sufficient conidia to test each isolate for the presence of the 26 remaining enzymes. For this reason, the study was separated into two major experiments. All isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis* listed in Table 1, with the exception of *P. sacchari* 21 and 23, were tested for the enzymes: AGP, FDP, β-GLU, GDH, GPI, GAPDH, PEP-LLL, PEP-PAP, PGD, and SOD. The remaining enzyme activities were analyzed in isolates of *P. sorghi*, *P. sacchari*, and *P. philippinensis* that produced sufficient conidia (Table 3). Each electrophoretic run was repeated at least two times. A previous study (4) showed that banding patterns did not vary with the age of the isolate, species of host, and the time of year the conidia were collected. Banding patterns are presented in Figures 1-4; the distribution of phenotypes is depicted in Table 3.

Simple matching coefficients were calculated for each pair of isolates based on the expression of 10 and 26 enzymes. Two sets of SSMs were calculated because of the differing sample sizes in the two experiments. The average SSMs calculated within and between species are presented in Table 4. Isolates of *P. sorghi*, *P.*

TABLE 3. Distribution of phenotypes by isolates of *Peronosclerospora sorghi*, *P. sacchari*, and *P. philippinensis*[a]

Species	Isolate no.	AGP[b]	FDP	GAPDH	GDH	β-GLU	GPI	PEP-LLL	PEP-PAP	PGD	SOD
P. sorghi	01	3	···	1	1	3	2	1	···	···	4
	02	3	···	···	1	1	1	1	···	1	4
	21	3	1	1	1	3	2	1	···	1	4
	22	3	···	···	1	1	3	···	···	1	4
	31	3	1	2	1	3	1	1	···	1	4
	32	3	···	···	1	2	1	···	···	1	4
	33	3	1	1	1	3	1	···	···	1	4
	34	3	1	1	1	2	1	···	···	1	4
	41	3	1	1	1	1	1	1	···	1	4
	42	3	1	···	1	3	1	···	···	1	4
	71	3	1	1	1	3	1	···	···	1	4
	72	3	1	1	1	1	2	1	···	1	4
	73	3	1	1	1	3	1	···	···	1	4
	81	3	···	1	1	···	1	···	···	···	4
Thailand	11	2	2	3	2	3	5	1	1	1	2
isolates	12	2	2	3	2	3	5	1	1	1	2
P. sacchari	01	1	3	4	1	2	4	1	2	1	3
	02	1	3	4	1	3	6	1	2	1	3
	03	1	3	4	1	3	6	1	2	1	3
	04	1	3	4	2	3	6	1	2	1	3
	05	1	3	4	2	3	6	1	2	1	3
	06	1	3	4	2	3	6	1	2	1	3
P. philippinensis	01	1	3	4	1	2	4	1	2	1	1
	02	1	3	4	1	2	4	1	2	1	1
	04	1	3	4	1	3	4	1	2	1	1
	06	1	3	4	1	3	4	1	2	1	1
	08	1	3	4	1	3	4	1	2	1	1
	11	1	3	4	1	3	4	1	2	1	1
	12	1	3	4	1	3	4	1	2	1	1
	14	1	3	4	1	3	4	1	2	1	1

[a] Phenotype number represents that depicted in Figure 1.
[b] Enzyme abbreviations spelled out in Table 2.

philippinensis, and *P. sacchari* all produced intraspecific SSMs between 0.75 and 1.00, representing a limited amount of intraspecific variation. Isolates of *P. sacchari* and *P. philippinensis* were closely related and probably represent a single species with SSMs ranging from 0.7 to 0.9. Isolates of *Peronosclerospora* from Thailand were not closely related to *P. sorghi*, *P. sacchari*, or *P. philippinensis*; the average SSMs with these species ranged between 0.1 and 0.3. Collections of the Thailand isolate made in 1975 (#11) and 1985 (#12) were identical.

In a separate experiment, the banding patterns of an isolate of *P. maydis* (#01) from West Java, Indonesia, were compared to those of *P. sorghi* (#01, 31, and 34), the Thailand isolates (#11 and 12), and *P. sacchari* (#01, 21, and 23) for 14 enzymes. The isolate of *P. maydis* shared phenotypes with both *P. sacchari* (AAT, AGP, GPI, MPI) and the Thailand isolates (AAT, β-GLU, MDH, GAPDH). It displayed unique phenotypes for the enzymes GR, IDH, PEP-GL, PEP-LLL, and XDH. *P. maydis* did not share any banding patterns with isolates of *P. sorghi*, except for the enzyme PGD that produced a single phenotype for all isolates studied (Table 3). A second isolate of *P. maydis* (#02) from East Java, Indonesia, was very limited in spore production and could only be tested for seven enzymes (AAT, GAPDH, GR, MDH, PEP-GL, PEP-LLL, and PGD). It produced banding patterns identical to *P. maydis* #01 in each case.

In an additional study, the banding patterns of two isolates of *B. lactucae* were compared with those from *P. sorghi* (#01, 02, 21, 31, 34, 41, 71, 72, 81), the Thailand isolates (#11, 12), *P. sacchari* (#01, 02, 03, 04, 05, 06), and *P. philippinensis* (#01, 02, 04, 06, 08, 11, 12, 14) for the enzymes AGP, FDP, GAPDH, GDH, β-GLU, G6PDH, GPI, GR, MADH, MDH, PGD, and SOD. No phenotypes were shared among isolates of *B. lactucae* and any *Peronosclerospora* species for 10 of 12 enzymes. The isolates of *B. lactucae* did share common phenotypes with *P. philippinensis* and *P. sacchari* for GPI (phenotype #4 in Fig. 1) and with the Thailand isolates for GAPDH (phenotype #3 in Fig. 1).

Banding patterns for 11 isolates of *Peronospora tabacina* were compared with a single isolate of *P. sorghi* (#44) and the Thailand (#11) for the enzymes AGP, GPI, GR, IDH, PEP-GL, PGD, and SOD. All isolates of *Peronospora tabacina* produced identical banding patterns for these seven enzyme systems. *Peronospora tabacina* and the Thailand isolate did not share any phenotypes for these seven enzymes. All isolates of *P. tabacina* produced multiple bands for IDH and shared one of these bands with *P. sorghi* #44 (Fig. 2). All three taxa could be easily differentiated by electrophoresis.

Aminopeptidase assays. Aminopeptidase profiles were constructed for isolates of *P. sorghi*, *P. philippinensis*, *P. sacchari*, and the Thailand isolate. Although some significant differences were detected in the levels of hydrolysis of the β-naphthylamine substrates, the differences did not correspond with taxonomic groupings. Aminopeptidase profiles could not be used to identify or distinguish species of *Peronosclerospora* due to these high levels of intraspecific variation. A representative aminopeptidase profile is presented in Figure 5.

DISCUSSION

Electrophoresis was used to clarify relationships among four members of the genus *Peronosclerospora*. The inability of certain isolates of *P. sorghi* to sporulate in large quantities under greenhouse conditions prevented a direct comparison of our 14 isolates of this species for all 26 enzymes. The comparisons that were made show that isolates of *P. sorghi* from different parts of the world could not be distinguished; banding patterns were shared by isolates from the Eastern and Western hemispheres. One exception was the banding patterns of the isolates from Thailand. Banding patterns of these isolates were strikingly different from those of other isolates of *P. sorghi*. The Thailand isolates were also distinct from isolates of *P. sacchari*, *P. philippinensis*, and *P. maydis* and probably represent another species. Separation from *P. sorghi* is supported by differences in host range (7), effect of temperature on sporulation and germination (5), symptomatology (7), conidial size (2), and the number of nuclei per conidiospore (2).

The two isolates from Thailand produced identical banding patterns for the 10 enzymes that were tested. This was unexpected since these collections were pooled field isolates collected at 10-yr intervals. The establishment of downy mildew infections from single conidia has not been successful to date. For this reason, all work was done with mass field collections. Multiple bands were observed in several enzyme systems (ACP, AK, DIA, EST, and

AC	ACP	AK	DIA	EST	GK	G6PDH	GPT	GR	IDH	MADH	MDH	MPI	PEP-GL	TPI	XDH
3	1	1	1	1	3	1	...	...	1	3	1	1	2	...	1
3	1	1	1	1	3	2	...	...	1	3	1	...	2	...	...
2	2	2	2	2	1	4	1	2	2	2	2	3	1	2	3
1	3	3	4	3	2	3	2	1	3	1	3	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	4	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	4	2	1	1	1
1	3	3	4	3	2	3	2	1	3	1	3	2	1	1	1
1	3	3	4	3	2	3	2	1	3	1	5	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	6	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	6	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	6	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	6	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	6	2	1	1	1
1	3	3	3	3	2	3	2	1	3	1	6	2	1	1	1

MDH) and may represent the expression of mixed genomes. Multiple bands can also be explained by the expression of different loci for an enzyme, heterozygosity, and the production of secondary isozymes (11).

Certain isolates of *P. sorghi* failed to produce bands for specific enzyme systems. It is unknown whether the absence of these bands is due to the expression of null alleles or from inadequate sample sizes. It would be necessary to perform crossing experiments to determine whether null alleles are involved, but crossing experiments are not currently possible within this genus. The incorporation of these missing bands into the SSMs may introduce some error into these calculations if null alleles are not involved, but the absence of these bands could not be ignored. Similar values for SSMs were obtained when those isolates with missing bands were eliminated from the calculation.

Simple matching coefficients between species were generally higher when 10 enzymes and 30 isolates were studied than when 26 enzymes were studied for 14 isolates. This is probably due to the greater percentage of enzymes with little or no variation in banding patterns (PGD, GDH, and PEP-LLL) in the former experiment. Intraspecific SSMs did not vary between the two studies.

Isolates of *P. sacchari* and *P. philippinensis* were shown to be similar by electrophoretic banding patterns. The average SSM between these species was 0.86 when 26 enzymes were compared; this value falls within the range expected for intraspecific variation (1). When Weston (26) described *P. philippinensis* (as *Sclerospora philippinensis* Weston), he stated that this organism may be conspecific with *P. sacchari*. According to Weston (26), the only differences between these species were the inabilities of *P. philippinensis* to infect sugarcane and form oospores. Bonde and Peterson (3) have shown that *P. philippinensis* can colonize sugarcane, so this objection has been removed, regardless of its validity as a species characteristic (7). Studies of symptom expression (7), host range (4), and response to environmental conditions (5) support this synonymy.

Many of the electrophoretic banding patterns of *P. maydis* were unique. This confirms the classification of this organism as a separate species. Isolates of *P. maydis* shared some electrophoretic phenotypes with the Thailand isolates and *P. sacchari* in certain enzyme systems and appear to be biochemically more similar to these organisms than to *P. sorghi*. More material of *P. maydis* is needed so that accurate intra- and interspecific SSMs can be calculated.

Certain inconsistencies were detected between this study and the initial report published by Bonde et al (3). Multiple bands were previously reported in the enzyme systems GPI and IDH; these were not detected in the current study. Different buffer systems were employed in the present study, which improved the resolution of the banding patterns. In most cases, direct comparisons of the banding patterns could not be made between the two studies since different buffer systems were employed.

Aminopeptidase activity could not be used to distinguish or identify the different species of *Peronosclerospora*. This is in contrast to a previous report (18) in which different levels of hydrolysis were detected among species. These interspecific differences were not consistent after multiple repetitions. Information from aminopeptidase profiles have been used to design artificial growth media for fastidious organisms (13,14). Studies are currently under way to determine whether this information can be used to develop such a medium for *Peronosclerospora*.

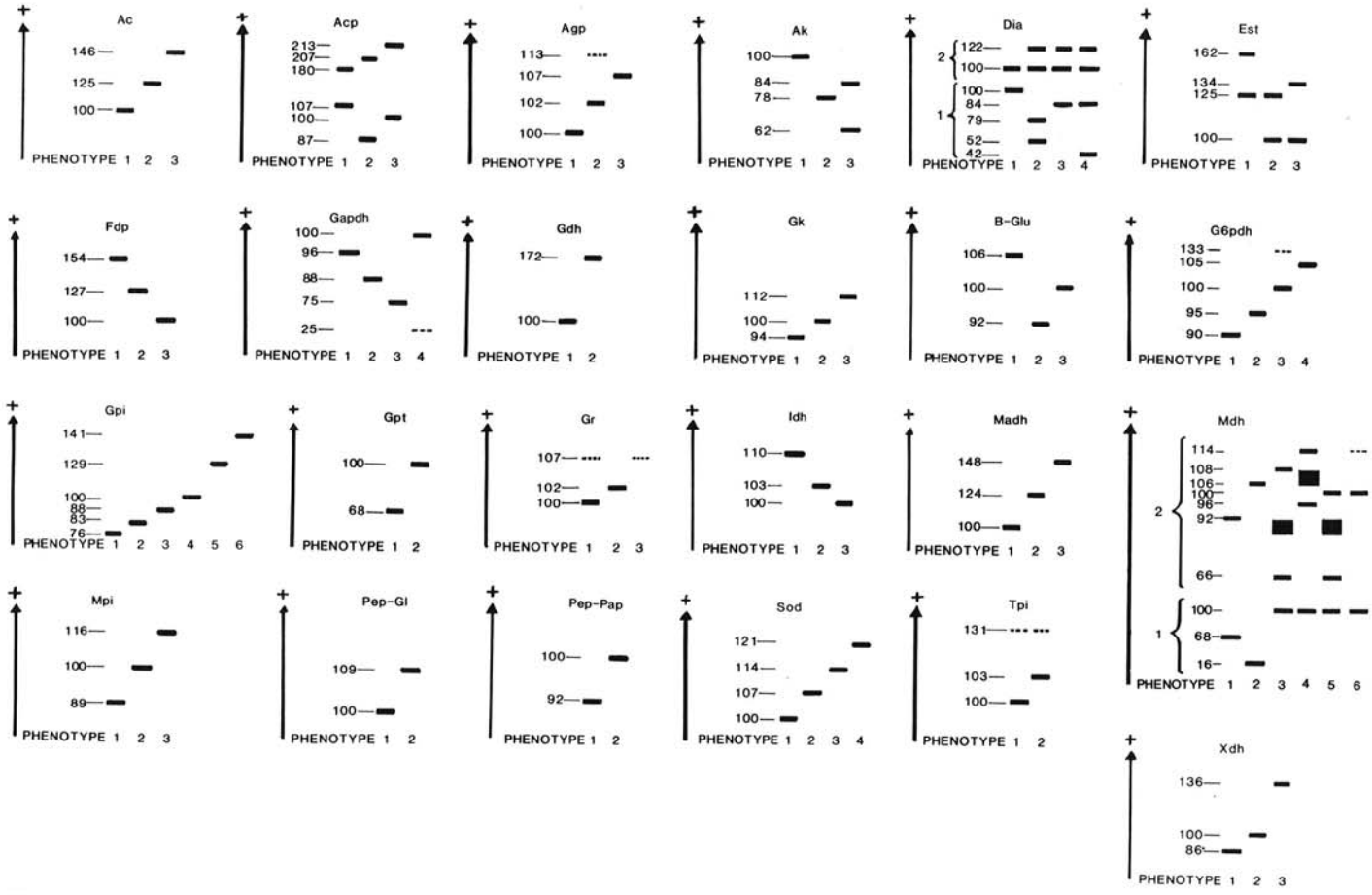
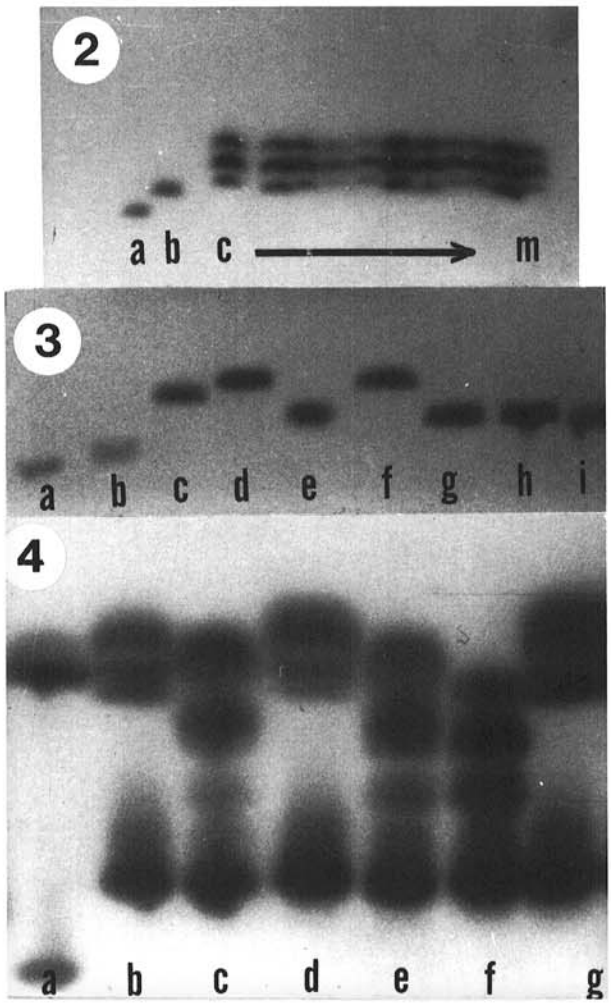


Fig. 1. Electrophoretic phenotypes for 26 enzymes. Numbers at right represent band designations. The most common band (i.e., that contained by the greatest number of isolates) was assigned an arbitrary designation of 100. The movement of all remaining bands is described relative to the movement of the most common band. An enzyme that migrated 30% further than the most common band would be designated 130. Dotted lines represent faint, secondary bands.



Figs. 2–4. 2, Distribution of banding patterns obtained for isocitrate dehydrogenase (IDH). Letters represent sample designations: a, Thailand isolate 11; b, *Peronosclerospora sorghi*; c–m, different isolates of *P. tabacina*. **3,** Distribution of banding patterns obtained for glucose phosphate isomerase (GPI). Letters represent sample designations: a, *P. sorghi* #31; b, *P. sorghi* #72; c, Thailand isolate #11; d, *P. sacchari* #02; e, *P. sacchari* #01; f, *P. sacchari* #03; g, *P. philippinensis* #01; h, *P. philippinensis* #02; i, *P. philippinensis* #03. **4,** Distribution of banding patterns obtained for malate dehydrogenase (MDH). Letters represent sample designations: a, Thailand isolate #11; b, *P. sacchari* #02; c, *P. sacchari* #01; d, *P. sacchari* #03; e, *P. philippinensis* #01; f, *P. philippinensis* #02; g, *P. philippinensis* #04.

TABLE 4. Average intra- and interspecific simple matching coefficients based on 10 and 26 enzymes

Intra- or interspecific comparison	Simple matching coefficient[a]	
	10 enzymes	26 enzymes
Peronosclerospora sorghi	0.75	0.77
Thailand isolates	1.00	...
P. sacchari	0.88	0.90
P. philippinensis	0.96	0.95
P. sorghi vs. Thailand isolates	0.17	0.10
P. sorghi vs. *P. sacchari*	0.21	0.15
P. sorghi vs. *P. philippinensis*	0.24	0.15
Thailand isolates vs. *P. sacchari*	0.33	0.14
Thailand isolates vs. *P. philippinensis*	0.27	0.14
P. sacchari vs. *P. philippinensis*	0.73	0.86

[a] Average simple matching coefficients were based on 10 and 26 enzymes due to the different sample sizes used in the two experiments. Ten enzymes were examined for all isolates listed in Table 1 with the exceptions of *P. sacchari* 21 and 23 and *P. maydis* 01 and 02. Twenty-six enzymes were studied for the isolates: *P. sorghi* 72, 31; Thailand isolate 11; *P. sacchari* 01, 02, 03; *P. philippinensis* 01, 02, 04, 06, 08, 11, 12, 14. Simple matching coefficients calculated as described by Sokal and Mitchener (22).

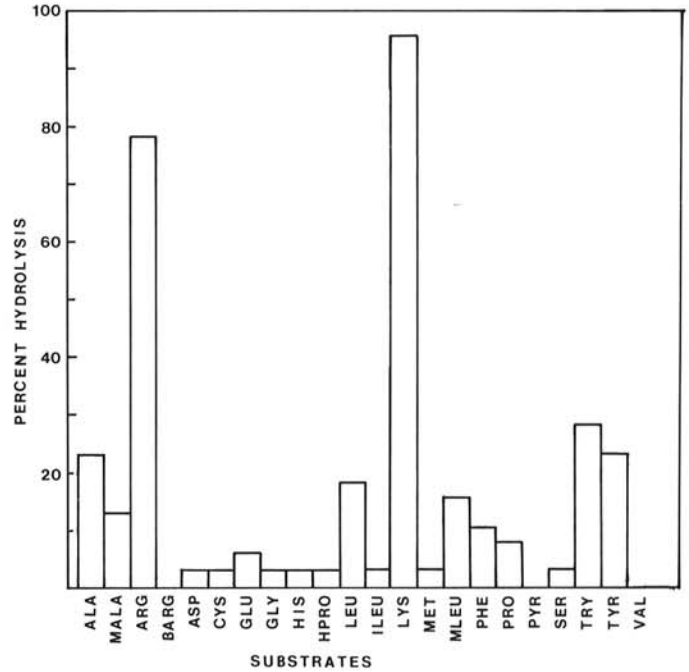


Fig. 5. Aminopeptidase profile of *Peronosclerospora philippinensis* #01 after 5 hr of incubation. Similar aminopeptidase profiles were obtained for isolates of *P. sorghi*, *P. philippinensis*, *P. sacchari*, and Thailand isolate (#11).

LITERATURE CITED

1. Avise, J. C. 1974. Systemic value of electrophoretic data. Syst. Zool. 23:465-481.
2. Bonde, M. R., Kenneth, R. G., Ben-Ze'ev, I. S., Peterson, G. L., Micales, J. A., and Farr, D. F. 1987. Morphological and Karyological Comparisons of *Peronosclerospora sorghi*, *P. sacchari*, and *P. philippinensis*. Final Report to United States-Israel Binational Agricultural Research and Development Fund, Grant Number US-563-82, Bet Dagan, Israel. 171 pp.
3. Bonde, M. R., and Peterson, G. L. 1983. Comparison of host ranges of *Peronosclerospora philippinensis* and *P. sacchari*. Phytopathology 73:875-878.
4. Bonde, M. R., Peterson, G. L., Dowler, W. M., and May, B. 1984. Isozyme analysis to differentiate species of *Peronosclerospora* causing downy mildews of maize. Phytopathology 74:1278-1283.
5. Bonde, M. R., Peterson, G. L., and Duck, N. B. 1985. Effects of temperature on sporulation, conidial germination, and infection of maize by *Peronosclerospora sorghi* from different geographical areas. Phytopathology 75:122-126.
6. Clayton, J. W., and Tretiak, D. N. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board Can. 29:1169-1172.
7. Duck, N. B., Bonde, M. R., Peterson, G. L., and Bean, G. A. 1987. Sporulation of *Peronosclerospora sorghi*, *P. sacchari*, and *P. philippinensis* on maize. Phytopathology 77:438-441.
8. Exconde, O. R., Elec, J. V., and Advincula, B. A. 1968. Host range of *Sclerospora philippinensis* Weston in the Philippines. Philipp. Agric. 52:175-188.
9. Exconde, O. R., and Raymundo, A. D. 1974. Yield loss caused by Philippine corn downy mildew. Philipp. Agric. 58:115-120.
10. Frederiksen, R. A., and Bockholt, A. J. 1969. *Sclerospora sorghi*, a pathogen of corn in Texas. Plant Dis. Rep. 53:566-569.
11. Harris, H., and Hopkinson, D. A. 1976. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland Publishing Co., Amsterdam.
12. Huber, D. M., Fortes, J. F., Pecknold, P. C., and Harmon, M. C. 1982. Aminopeptidase profiles of four species of *Phytophthora*. Appl. Environ. Microbiol. 43:597-602.
13. Lee, K. L., Buckley, H. R., and Campbell, C. C. 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. Sabouraudia 13:148-153.
14. Lee, K. L., Reca, M. E., and Campbell, C. C. 1975. Amino acid synthetic media for fungal pathogens based on aminopeptidase specificities: *Histoplasma capsulatum*, *Blastomyces dermatitidis*,

Paracoccidioides brasiliensis, and *Cryptococcus neoformans*. Sabouraudia 13:142-147.
15. Leu, L. S. 1973. Effects of temperature on conidial size and sporulation of *Sclerospora sacchari*. Plant Prot. Bull. Taiwan 15:106-115.
16. Micales, J. A., Bonde, M. R., and Peterson, G. L. 1986. The use of isozyme analysis in fungal taxonomy and genetics. Mycotaxon 27:404-449.
17. Micales, J. A., Bonde, M. R., Peterson, G. L., and Fry, W. E. 1985. Differentiation of *Peronosclerospora* species by isozyme analysis. (Abstr.) Phytopathology 75:1339.
18. Micales, J. A., Huber, D. M., Bonde, M. R., Peterson, G. L., and Fry, W. E. 1985. Aminopeptidase activity of *Peronosclerospora sorghi*, *P. sacchari*, and *P. philippinensis*. (Abstr.) Phytopathology 75:627.
19. Pupipat, U. 1976. Corn downy mildew research at Kasetsart University. Kasetsart J. 10:106-110.
20. Ridgway, G. J., Sherburne, S. W., and Lewis, R. D. 1970. Polymorphisms in the esterases of Atlantic herring. Trans. Am. Fish. Soc. 99:147-151.
21. Schmitt, C. G., and Freytag, R. E. 1977. Response of selected resistant maize genotypes to three species of *Sclerospora*. Plant Dis. Rep. 61:478-481.
22. Sokal, R. R., and Michener, C. D. 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. 38:1409-1438.
23. Sun, M. H., Chang, S. C., and Tseng, C. M. 1976. Research advances in sugarcane downy mildew of corn in Taiwan. Kasetsart J. 10:89-93.
24. Warren, H. L., Jones, A., Jr., and Huber, D. M. 1977. Morphological and physiological differences between *Bipolaris maydis* races O and T. Mycologia 69:773-782.
25. Westley, J. W., Anderson, P. J., Close, V. A., Halpern, B., and Lederberg, E. M. 1967. Aminopeptidase profiles of various bacteria. Appl. Microbiol. 15:822-825.
26. Weston, W. H., Jr. 1920. Philippine downy mildew of maize. J. Agric. Res. 19:97-122.
27. Weston, W. H., Jr. 1923. Philippine *Sclerosporas* of maize. J. Agric. Res. 23:239-278.

to the currently described species have been reported (25,29,30).