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SEROLOGICAL STUDY OF SAPROPHYTIC STRAINS OF *FUSARIUM OXYSPORUM* AND *F. OXYSPORUM* F. SP. *ELAEIDIS*

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(With 2 Text-figures)

Strains of *F. oxysporum* Schlecht. f. sp. *elaeidis* Toovey and of *F. oxysporum* Schlecht. isolated from soil are analysed serologically by the double diffusion technique in agar gel and by immunoelectrophoresis. The great variability in the antigenic constitution of extracts of strains within a phenotype or within a pathogenic forma renders the distinction of the different types impossible with serological techniques. All the strains studied, isolated from a restricted geographical area, seem to belong to one serological group.

In the course of the investigation on the development of the population of *F. oxysporum* Schlecht. in the soil in relation to the vascular wilt disease of the oil palm (*Elaeis guineensis* Jacq.), three different wild types were isolated (Renard, 1967): type FO1 rare and limited to the savannah, type FO4 found in all the soils analysed and type FOS isolated abundantly from the palm soils. Pathogenic strains FO isolated from diseased trees are of the FOS phenotype. FO and FOS are sporodochial types while FO1 and FO4 are mycelial types differing in their pigmentation and the abundance of aerial mycelium.

The symptoms of vascular wilt may appear from the third year, at random, in plantations established on savannah soils.

It is thus interesting to know if there exists any relationship between the different types of *Fusarium*, from the saprophytic types of the savannah soil and the pathogenic form isolated from the oil palm. Meyer & Renard (1969), employing protein and esterase spectra obtained by polyacrylamide gel electrophoresis, have shown great biochemical heterogenicity within pathogenic formae even from restricted geographical areas. This technique seems thus inadequate for showing any relationship between our different isolates or for differentiating pathogenic forms from saprophytic ones. Madhosingh (1964), Morton & Dukes (1966) and Kalyanasundaram, Desai & Sirsi (1967) were able to distinguish by serological techniques species within the genus *Fusarium*, and formae speciales and races within *F. oxysporum*. We therefore used immunochemical techniques to approach the problem of the relationship between the different types of *F. oxysporum* as cited above.

MATERIAL AND METHODS

The origin of strains, their phenotypes and their corresponding antisera are given in Table 1.

All the strains were isolated from the region of Dabou (Ivory Coast). In some experiments we included *F. oxysporum* Schlecht. f. sp. *melonis* Snyder & Hansen (M15) and *F. solani* (Mart.) Sacc. (FS).

Table 1. *Origin, type and corresponding antisera of the strains of* Fusarium oxysporum *studied*

Strains	Types	Corresponding antisera
R1*	FO	A
R20*	FO	E
R21*	FO	F
R23*	FO	G
R25*	FO	H
R13†	FOS	B
R14‡	FO1	C
R16‡	FO4	D

* *F. oxysporum* Schlecht. f. sp. *elaeidis* Toovey.
† *F. oxysporum* Schlecht. from palm tree plantation soil.
‡ *F. oxysporum* Schlecht. from savannah soil.

The isolates were grown at 28 °C in the dark, in 500 ml Erlenmeyer flasks with 100 ml of Richards medium. After 9 days incubation, the cultures were filtered and the mycelium washed and homogenized for 3 min with 0·1 M phosphate buffer (pH 7·1) in a Waring blendor. This homogenate was ground in a Braun cell disintegrator in which it was mixed with 0·2–0·3 mm diam glass beads in the proportion of 1:1 (v/w). The containers were shaken mechanically for 4 min, being placed in a metal cylinder whose axis described a circular movement at a speed of 4,000 rev./min. whilst cooled by a stream of carbon dioxide.

The antisera were obtained by intramuscular injection of the homogenate emulsified into an equal volume of Freund's incomplete adjuvant. The injections were given twice a week and continued for five consecutive weeks. The sera were collected 8 days after the final injection. They were lyophilized or conserved at −25°.

The antigens used in the serological tests were obtained by centrifuging the homogenate at 3800 ***g***; the soluble proteins in the supernatant were precipitated with saturated ammonium sulphate, centrifuged at 20000 ***g***, dialysed overnight against veronal buffer 0·025 M (pH 8·6) then resuspended in the same buffer. The protein content was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951). A final concentration of 4500 to 6000 μg of proteins per ml was used in the serological tests.

The agar double-diffusion method was used to compare antigen-antibody reactions following the standard Ouchterlony method. The diameter of the holes was 5 mm and the distance between the holes from

centre to centre 10 mm. The agar plates were maintained at 25° and the results observed after 20 and 40 h reaction.

Immunoelectrophoretic analysis was done on 25 × 75 mm glass slides covered with 4 ml of a 1% agarose reinst (Serva) solution in veronal buffer, 0·025M (pH 8·6), during 90 min at 4·5 V/cm. The development of patterns by diffusion of antiserum was continued up to 48 h at room temperature.

Comparison of antigen-antibody reactions was based on the number, position and intensity of the precipitation arcs.

RESULTS

Double diffusion in agar gel

Antiserum A reacts with its homologue antigen R1, giving at least ten precipitation lines, but this result is not constant throughout. With different extracts of R1 or with extracts of other strains of the same phenotype the number of lines varies. In cross reaction of antiserum A against the antigens FOS, FO1 and FO4, different number of lines are detected; between different strains of the same phenotype, the number of lines is not constant.

Table 2. *Maximum number of precipitation lines obtained in agar plates with antiserum A reacting against antigens of* F. oxysporum *saprophytic types* (*FOS*, *FO*1, *FO*4), F. oxysporum *f. sp.* elaeidis (*FO*), F. oxysporum *f. sp.* melonis (*M*15), *and* F. solani (*FS*)

Types and species	Precipitation lines									
	a	b	c	d	e	f	g	h	i	j
FO	+	+	+	+	+	+	+	+	+	+
FOS	+	.	.	+	+	+	+	+	+	+
FO1	+	+	+	.	+	+	+	+	+	+
FO4	.	.	+	+	+	+	+	.	+	+
M15	.	.	+	.	.	+	+	+	+	+
FS	.	.	+	.	.	.	+	.	+	+

The isolates from the Ivory Coast have five antigens in common; *F. oxysporum* f. sp. *melonis* shares four antigens with them and *F. solani* only three.

Within the forma *elaeidis* (FO type), the differences are as great as those existing between the different types studied. They are not only represented by different numbers of lines but also by differences in their intensity and clarity which suggests that the concentration for individual antigens is highly variable within a strain as well as between strains of the same phenotype.

These results are partially confirmed by the absorption experiments. The mixing of antiserum A and its antigen FO in equal proportion is not sufficient to absorb the antiserum completely, but when one part of antiserum is mixed with three parts of antigen, the antiserum is completely absorbed by FO and FOS; when absorbed with FO4, it reacts against FOS and FO, giving two precipitation lines, and when absorbed with

FO 1 three arcs with FO antigen, two with FOS and one with FO 1 and FO4. Figure 1 shows the result of one experiment.

The macromolecular fraction of FO and FOS extracts as defined by Gooding (1966) for *Fomes annosus*, reacts with the antiserum A giving four arcs, while those of FO 1 and FO4 give only three arcs respectively.

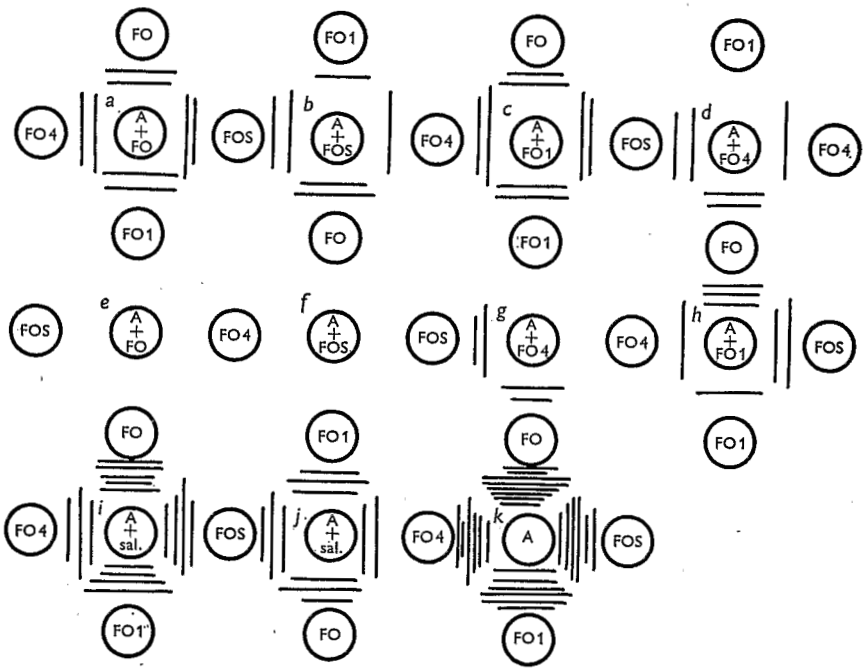

Fig. 1. Diagrams of serological reactions with antiserum A absorbed with extracts of the different phenotypes or diluted with physiological saline in different proportions. a–b–c–d, one part antiserum mixed with one part antigenic extract; e–f–g–h, one part antiserum mixed with three parts antigenic extract; i, one part antiserum mixed with one part physiological saline; j, one part antiserum mixed with three parts physiological saline; k, serum A undiluted.

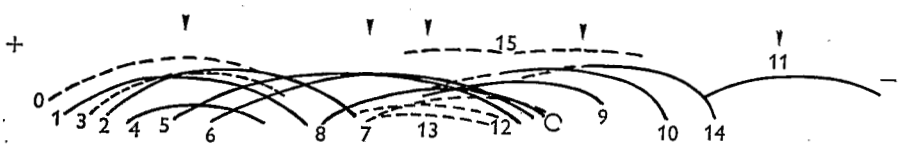

Fig. 2. Schematic reconstitution of the immunoelectrophoretic analysis of antiserum A against FO antigens.

Immunoelectrophoresis

The immunoelectrophoresis of FO antigens against antiserum A gives 16 precipitation lines which may be grouped into five zones (Fig. 2). Towards the anode there exist three zones of higher protein concentration. The most distant one contains the lines 0, 1, 2, 3 and 4; lines 5 and 6

correspond to a zone of average migration, these lines are close to each other and wide spread; lines 7, 8, 12 and 13 belong to the group of slow electrophoretic migration. Lines 9, 10, 14 and 15 have sometimes no migration or a very slight one towards the cathode. Lastly, line 11 migrates typically towards the cathode. Lines 0, 3, 7, 12, 13 and 15 are always faint and it is among them that the greatest variability exists. This is apparent in different extracts from the same isolate as well as between extracts of isolates of different phenotypes.

All the antisera share at least the antibodies 0, 1, 2, 4, 5, 6, 9, 10, 11, 15 (Table 3) corresponding to the presence of common molecular constituents in the antigenic extracts (Table 4).

Table 3. *Constitution of the antisera revealed by the cross-reaction of each serum against the different antigens from saprophytic strains of* F. oxysporum *and strains of* F. oxysporum *f. sp.* elaeidis

	Precipitation lines															Total number of anti-
Antisera	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	bodies
A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	.	+	15
E	+	+	+	.	+	+	+	.	.	+	+	+	.	.	.	+	10
F	+	+	+	+	+	+	+	.	+	+	+	+	.	.	.	+	12
G	+	+	+	+	+	+	+	.	+	+	+	+	.	.	+	+	13
H	+	+	+	.	+	+	+	.	.	+	+	+	.	.	+	+	11
B	+	+	+	.	+	+	+	+	+	+	+	+	.	+	.	+	13
C	+	+	+	.	+	+	+	.	.	+	+	+	.	.	.	+	10
D	+	+	+	.	+	+	+	+	+	+	+	.	.	.	+	+	13

Table 4. *Maximum number of antigenic constituents detected in the different phenotypes of* F. oxysporum

	Precipitation lines															Total number
Pheno-																of
types	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	antigens
FO	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16
FOS	+	+	+	.	+	+	+	+	+	+	+	+	.	+	.	+	13
FO1	+	+	+	.	+	+	+	.	.	+	+	+	.	.	+	+	11
FO4	+	+	+	.	+	+	+	+	+	+	+	+	+	.	+	+	14

Table 5. *Number of precipitation arcs detected in cross-reactions between the antisera and the extracts of the different strains each representing a phenotype of* F. oxysporum

	Phenotypes			
Antisera	R1 (FO)	R13 (FO3)	R14 (FO1)	R16 (FO4)
A	*15*	10	7	10
E	10	5	8	8
F	12	4	6	8
G	12	6	5	8
H	10	5	7	8
B	12	*11*	7	9
C	7	5	*8*	9
D	9	7	10	*12*

The italicized values represent the number of lines developed by an antiserum against its homologue antigen.

Table 3 shows how heterogeneous are the antisera of the different strains of *F. oxysporum* f. sp. *elaeidis*, the number of arcs developed against the antigens of this type varying from 10 to 15.

In Table 5 are collected all the results of the cross-reactions of the different antisera against the antigens of four strains, each representing a phenotype. The results show that the number of precipitation lines is not necessarily the highest when an antiserum reacts against its homologue antigen.

DISCUSSION

Immunoelectrophoresis allows a better separation and the detection of a greater number of antigens than the double diffusion in agar plates. However, the complex constitution of antigenic extracts, the heterogenicity of the antisera inherent to the differential animal response and the very low titre of the antisera (no reaction occurs with any dilution above 1:8) nevertheless limit the amelioration expected from this method.

Within a strain, electropherograms are not exactly identical from one culture to another one, the variations bearing as well on the number of arcs as on the intensity of their specific precipitate. Between the strains the same variability is encountered and is not greater between the isolates of different types than within a type.

Isolates FO, FOS and FO4 possess 12 molecular constituents in common. The differences between the isolates are situated at the level of the lines 3, 7, 8, 12, 13, 14. They may be of qualitative or of quantitative nature, i.e. that the absence of a line does not mean necessarily that the molecular constituents are absent, but only at a level not detectable. This variability does not allow the separation of the types on the base of these observations, and does not confirm the gross cultural differences among the types. Between the isolates FO1 and the others, the serological relation seems to be more distant but not stable enough to differentiate them only on this basis.

Data reported in this paper reveal no major qualitative difference between saprophytic and pathogenic strains and between strains of different wild types. We may therefore regard all our isolates as belonging to the same serological group. Other authors have, however, distinguished taxonomic entities with serological techniques. Suerth & Varney (1968) claimed to differentiate several races of *Phytophthora fragariae* and Cheung & Marshall (1967) to characterise isolates of *Trametes* spp. In the latter case differences were obtained only with one antiserum and a limited number of cultures. In the same way, Madhosingh (1964), Kalyanasundaram *et al.* (1967) and Morton & Dukes (1966) could differentiate respectively species of *Fusarium* and pathogenic forms and races of *F. oxysporum* but these authors limited their investigations to one strain per taxa.

Meyer & Renard (1969) have shown with protein spectra obtained by electrophoresis on polyacrylamide gel that within pathogenic formae there exist great differences between the strains although separate entities can be distinguished. The discriminating power of the protein spectra revealed with amido-black seems thus to be much better than that of the spectra

obtained by immunoelectrophoresis. These authors obtained up to 25 protein bands on acrylamide gels instead of 16 antigenic compounds obtained by immunoelectrophoresis.

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