Neth. J. Pl. Path. 79 (1973) 70–80

# Differences in disc gel electrophorese pattern of soluble proteins excreted by different physiological races and isolates of Cladosporium fulvum

A. VAN DIJKMAN, S. J. DIELEMAN and A. KAARS SIJPESTEIJN

Institute for Organic Chemistry TNO, Biochemical and Microbiological Department, Utrecht, the Netherlands

Accepted 23 October 1972

**Abstract**

The extracellular soluble proteins of 15 different isolates comprising 9 different physiological races of *Cladosporium fulvum* were harvested from 3 to 4 weeks old shake cultures. The culture filtrates were purified by gel filtration over Sephadex G–25 and the $V_0$-fractions were freeze-dried. These preparations were submitted to disc gel electrophoresis on polyacrylamide gels at pH 9.5 at circa 4 °C.

The electrophoretical protein patterns appeared to differ for each isolate irrespective of the physiological race to which they belong. The various isolates originated from different sites in the Netherlands.

## Introduction

Disc gel electrophoresis of soluble proteins of fungal species has been employed successfully as a diagnostic and taxonomic aid by several phytopathologists and mycologists. It proved more convenient than serology, although the latter has other advantages. The high resolution obtained by the disc electrophoresis technique gives a complex but characteristic pattern of protein bands.

Investigations in the taxonomic field were carried out within the genera *Phytophthora* (Clare, 1963; Gill and Powell, 1963; Hall et al., 1970), *Septoria* (Durbin, 1966) and *Ceratocystis* (Stipes, 1967). Gill and Powell (1968) obtained disc gel protein patterns of 17 isolates and 8 physiological races of *Phytophthora fragariae*. These were nearly identical. Races or biotypes of *P. fragariae* could not be differentiated. Neither did Shipton and Fleischmann (1969) find differences in the protein patterns obtained by disc gel electrophoresis on polyacrylamide of extracts of soluble proteins from uredospores of *Puccinia coronata* f. *avenae*.

On the contrary Macko et al. (1967) claimed that the electrophoretical patterns of uredospore protein of two physiological races of *Puccinia graminis* differed significantly. Recently, electrophoretical separation of proteins in polyacrylamide gels was also useful in differentiating two pathotypes of *Heterodera rostochiensis* (Trudgill and Carpenter, 1971).

No such experiments have been carried out so far with the various physiological races of *Cladosporium fulvum*. In a study of the biochemical mechanism of the gene for gene relation between tomato and this pathogen we have found that the differences between the physiological races depend on the presence or absence of certain extra-

cellular high molecular products (Van Dijkman and Kaars Sijpesteijn, 1971, 1973). Hence it was thought of interest to compare the protein patterns obtained by disc gel electrophoresis of the extracellular high molecular fractions of the various physiological races.

## Materials and methods

*Fungal material.* The different physiological races of *C. fulvum* were obtained from the Institute of Phytopathological Research in Wageningen.

The cultures of monoconidial isolates were kept on potato-dextrose-agar slants at 24 °C. Shake cultures were prepared by inoculation of eight 1 liter-flasks each containing 125 ml of the following medium: 2% glucose, 0.2% casamino acids (Difco, technical grade), 2 ppm $ZnCl_2$, 2 ppm $MnSO_4.4H_2O$, 0.15% $MgSO_4.7H_2O$, 0.01% KCl, 0.0025 M $K_2HPO_4/KH_2PO_4$ (pH 6) in tap water. Each flask was inoculated with the spores of one 3 to 4 weeks old agar slant. The cultures were incubated for 3 to 4 weeks at 24 °C.

The identity of the physiological races was checked by inoculating appropriate tomato varieties. Results of these tests confirmed the disease pattern given by Kooistra (1964) and Hubbeling (1968). Re-isolations were carried out to obtain fresh, virulent cultures and these were lyophilized in skim milk at the Centraal Bureau voor Schimmelcultures at Baarn (the Netherlands) and stored in the refrigerator. The races will be indicated by their presumed avirulence genes (Van Dijkman and Kaars Sijpesteijn, 1971). The isolates used throughout this study are listed in Table 1.

Table 1. The physiological races, c.q. isolates, of *C. fulvum* used in the present investigation.

| Indices[1] | Genotype for avirulence | Locality of isolation | Province |
|---|---|---|---|
| 0 | $A_1A_2A_3A_4$ | Arcen* | Limburg |
| 0 | $A_1A_2A_3A_4$ | Dordrecht | Zd-Holland |
| 1 | $a_1A_2A_3A_4$ | Naaldwijk* | Zd-Holland |
| 2 | $A_1a_2A_3A_4$ | Sappemeer* | Groningen |
| 3 | $A_1A_2a_3A_4$ | Utrecht* | Utrecht |
| 4 | $A_1A_2A_3a_4$ | Den Hoorn | Zd-Holland |
| 4 | $A_1A_2A_3a_4$ | Naaldwijk | Zd-Holland |
| 4 | $A_1A_2A_3a_4$ | Naaldwijk* | Zd-Holland |
| 1.2 | $a_1a_2A_3A_4$ | Enkhuizen* | Nd-Holland |
| 1.2.3. | $a_1a_2a_3A_4$ | unknown | |
| 1.2.4 | $a_1a_2A_3a_4$ | Drunen | Nd-Brabant |
| 1.2.4 | $a_1a_2A_3a_4$ | Wilnis* | Utrecht |
| 1.2.4 | $a_1a_2A_3a_4$ | Wellerlooi | Limburg |
| 2.3.4 | $A_1a_2a_3a_4$ | Naaldwijk | Zd-Holland |
| 2.3.4 | $A_1a_2a_3a_4$ | Bergschenhoek* | Zd-Holland |

[1] Indices as given by Kooistra (1964) and Hubbeling (1968).

* These races were used in our leakage experiments (Van Dijkman and Kaars Sijpesteijn, 1971, 1973).

*Tabel 1. De voor het onderzoek gebruikte fysio's, c.q. isolaten van C. fulvum*

Of certain genotypes for avirulence different isolates were available originating from locations in the Netherlands that sometimes were more than 100 km apart.

*Gel filtration of culture filtrates.* To separate low and high molecular weight materials from the filtrates of the shake cultures, gel filtration on Sephadex G-25 (medium grade) was performed. In this process the culture filtrate was applied to a column of 3.8 × 91 cm; for larger quantities we used the K 100/100 column of Pharmacia (Uppsala, Sweden), also filled with Sephadex G-25 medium grade. The void volumes were determined with Blue Dextran 2000. The maximum sample volume for the small column was 300 ml ($V_t$ 1030 ml); for the K 100/100 columns, we applied mostly 1200 ml of culture filtrate; this column was also used to standardize combined G-25 void volume ($V_o$)-fractions of different batches of one isolate. Gel filtration was performed at about 4 °C with glass bidistilled water. Absorption at 280 nm of the effluent was recorded with a Uvicord 2 (LKB, Stockholm, Sweden). The fractions were collected either by hand or by an LKB 7000 Ultro Rac fraction collector placed in a Colora cool-box at approx. 2–4 °C, and freeze-dried (Cenco-Virtis freeze-drier, Gardiner, USA). Protein was determined by the Folin method (Lowry et al., 1951).

*Electrophoresis of the extracellular soluble proteins.* Disc gel electrophoresis of the extracellular soluble proteins was performed according to Ornstein (1964) and Davis (1964) with slight modifications in the procedure.

The polyacrylamide gels were prepared in running tubes 5 mm I.D. × 75 mm as described by Davis (1964), except that we used the persulphate catalyst system for both spacer and separation gels. The concentration of the acrylamide was 7.5%. Electrophoresis was performed at pH 9.5 and at about 4 °C in a commercial apparatus (Shandon Scientific Company, London) during 2.5 min at 1 mA per gel and subsequently for 45 min at 5 mA per gel.

Samples from the different freeze-dried high molecular preparations were dissolved in the buffer solution of the spacer gel and centrifuged at 10,000 g for 10 min at 4 °C. Aliquots from 5 to 100 µl of the supernatant containing 0.1 to 2.3 mg protein were applied on top of the spacer gels. Due to variation in the viscosity of the solutions, it was impossible to apply the same concentration of protein for each of the different races.

No front marker was used because a magenta-coloured pigment running with the front was functioning as such. The gels were removed from their running tubes by irrigation with a needle (Maurer, 1968). After fixation in 12.5% trichloroacetic acid the protein bands were stained with Coomassie Brilliant Blue R 250 in 12.5% trichloroacetic acid as described by Chrambach et al. (1967). To diminish the background effect of this staining procedure the gels were destained overnight in 10% trichloroacetic acid before scanning. It is known that the persulphate catalyst can cause artefacts (Loening, 1967). Therefore, we tested the gels after polymerization was complete. When immersed in a 2% solution of benzidine in 10% acetic acid, no blue colour developed. Obviously, no persulphate was present when electrophoresis was performed (Bennick, 1968).

Densitometer tracings of the gels were obtained by using a Photovolt model 520-A densitometer with reduced slitwidth, equipped with a Varicord 43 linear/log recorder. A red filter with a maximum transmittance at 610 nm was used for these scans. In

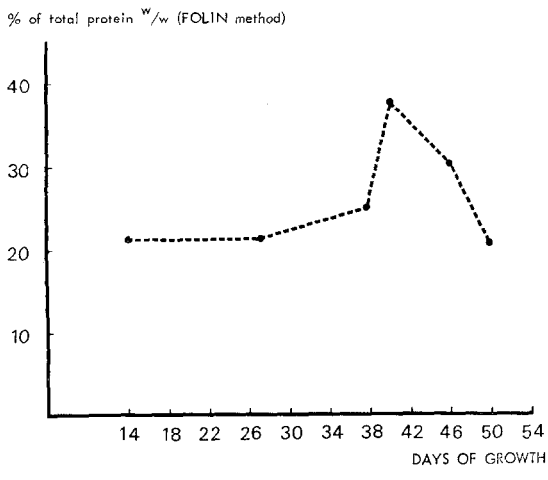


Fig. 1. Relation between growth period and total protein content of the freeze-dried $V_o$-fraction of culture filtrate of *C. fulvum* race $a_1A_2A_3A_4$.

*Fig. 1. De relatie tussen groeiduur en eiwitgehalte van de drooggevroren $V_o$-fractie van het cultuurfiltraat van C. fulvum fysio $a_1A_2A_3A_4$.*

later experiments we used a Beckman DU spectrophotometer, equipped with a Gilford Gel Scanner at 583 nm. Scans were recorded on a Servogor compensation recorder RE 514.

*Chemicals.* Acrylamide – Cyanogum 41, British Drug Houses – and N, N'-methylene-bis acrylamide – BIS, British Drug Houses – were purified by recrystallization from chloroform at 50 °C and acetone at 40 °C respectively, according to Loening (1967). Other chemicals used were all reagent grade.

## Results

*High molecular weight fractions of culture filtrates of C. fulvum.* The 15 isolates of *C. fulvum* comprising 9 different physiological races are given in Table 1.

The production of extracellular protein in shake culture was followed during 50 days after inoculation of the medium. The percentage of total protein present in the freeze-dried $V_o$-fraction of the culture filtrate of race $a_1A_2A_3A_4$ after 14, 27, 38, 40, 46 and 50 days respectively, is represented in Fig. 1. The graph indicates that after about four weeks of incubation abnormal increase in protein concentration took place, probably due to autolysis. In the experiments described below, cultures with an incubation period of 3 to 4 weeks were applied.

The elution patterns of gel filtration of the crude culture filtrates over Sephadex G-25 medium grade showed no essential differences between isolates examined. Such an elution pattern is given in an earlier publication (van Dijkman and Kaars Sijpesteijn, 1971). Also the pattern of the second purification over Sephadex G-25 was largely the same for the different isolates (Fig. 2).

*Electrophoresis.* The use of sucrose on the spacer gel was not required because the high viscosity of the solution prevented diffusion of the sample into the electrode buffer.

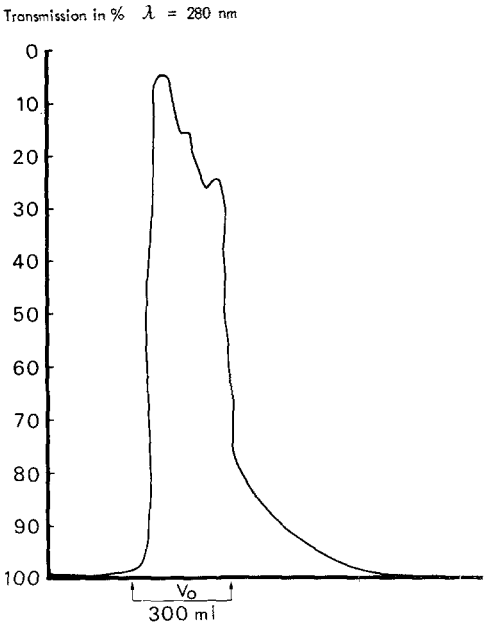


Fig. 2. Elution pattern of Sephadex G-25 gel filtration on a K 100/100 column of the combined $V_o$-fractons of *C. fulvum* race $A_1a_2A_3A_4$.

*Fig. 2. Elutiepatroon van een Sephadex G-25 gelfiltratie op een K 100/100 kolom van de gecombineerde $V_o$-fracties van C. fulvum fysio $A_1a_2A_3A_4$.*

At the electrophoretical front we always found a magenta-coloured band at the pH used (pH 9.5). The band coloured orange when the gel was placed in 1 N HCl. Small amounts of the anthraquinone cladofulvine isolated from *C. fulvum* applied to the system under the same electrophoretical conditions had the same electromobility and colour as the coloured band in our $V_o$-fraction of *C. fulvum* culture filtrates. In 1 N HCl cladofulvine colours orange. These findings suggest that the coloured band is cladofulvine or at least contains cladofulvine.

UV light absorption at 280 nm after fixation for 30 min in 12.5% trichloroacetic acid showed no distinct peaks.

Staining the gels with amido blue-black according to the method of Davis (1964) revealed only a few weak bands, but the staining procedure of Chrambach et al. (1967) resulted in clear, well reproducible protein patterns.

No protein bands could be detected after electrophoresis with reversed current. Apparently, all proteins had moved into the separation gel under the electrophoretical conditions used.

Figs. 3–11 show the electrophoretical protein patterns of eleven isolates of *C. fulvum* belonging to nine different physiological races. Obviously each isolate has a different pattern irrespective of the physiological race to which they belong. The patterns were well reproducible and when applied at various concentrations and at volumes from 5–100 µl the preparations yielded no qualitative variations. However, when samples were too concentrated their high viscosity caused disturbances in the spacer gel. Similar experiments were carried out with the isolates Dordrecht (0), Naaldwijk*(4), Wellerlooi (1.2.4) and Naaldwijk (2.3.4). Also their protein patterns were different again.

Fig. 3–10. Electropherograms of soluble extracellular proteins of different physiological races of *C. fulvum*. Electrophoresis was performed during 45 min, 5 mA/gel in 7.5% acrylamide gels at pH 9.5, temperature about 4°C. The gels were stained with Coomassie Brilliant Blue R 250 and scanned at 583 or 610 nm after one day.

*Fig. 3–10. Electroferogrammen van de oplosbare extracellulaire eiwitten van verschillende fysio's van C. fulvum. Elektroforese werd uitgevoerd met 5 mA/gel en 7,5% acryl amide gels by pH 9,5 gedurende 45 min en ongeveer 4°C. De gels werden gekleurd met Coomassie Brilliant Blue R 250 en gemeten bij 583 of 610 nm na 1 dag.*

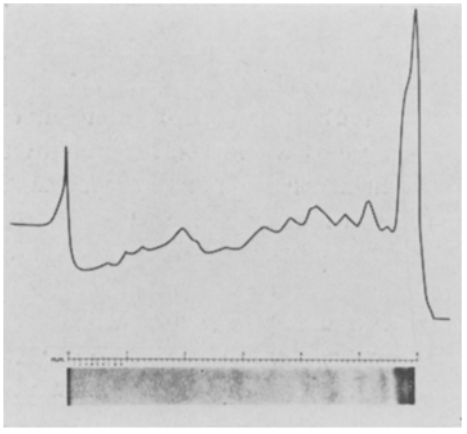

Fig. 3. 50 µl aliquot with 0.145 mg protein of physiological race $A_1A_2A_3A_4$, isolate from Arcen.

*Fig. 3. 50 µl monster met 0,145 mg eiwit van fysio $A_1A_2A_3A_4$, isolaat uit Arcen.*

Fig. 4. 20 µl aliquot with 0.094 mg protein of physiological race $a_1A_2A_3A_4$, isolate from Naaldwijk.

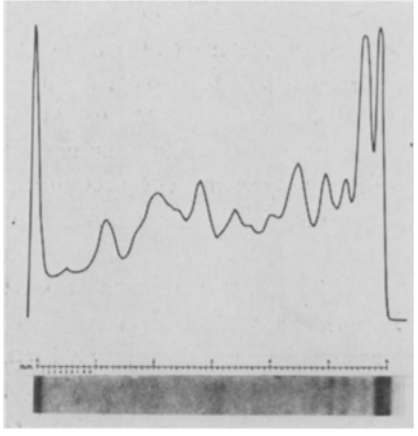

*Fig. 4. 20 µl monster met 0,094 mg eiwit van fysio $a_1A_2A_3A_4$, isolaat uit Naaldwijk.*

Fig. 5. 10 µl aliquot with 0.025 mg protein of physiological race $A_1a_2A_3A_4$, isolate from Sappemeer.

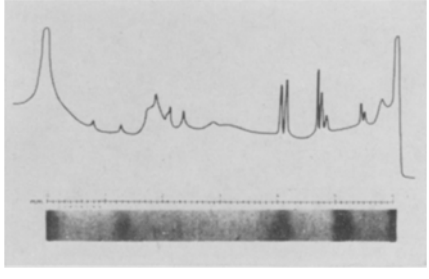

*Fig. 5. 10* µl monster met 0,025 mg eiwit van fysio $A_1a_2A_3A_4$, isolaat uit Sappemeer.

Fig. 6. 10 µl aliquot with 0,025 mg protein of physiological race $A_1A_2a_3A_4$, isolate from Utrecht.

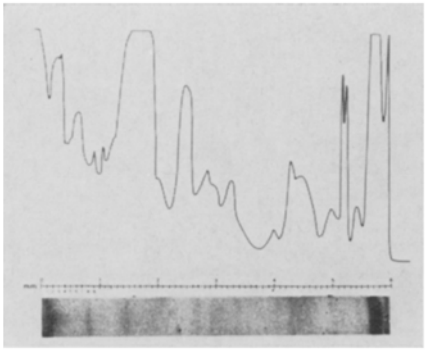

*Fig. 6. 10 µl monster met 0,025 mg eiwit van fysio $A_1A_2a_3A_4$, isolaat uit Utrecht.*

Fig. 7. 25 µl aliquot with 0.094 mg protein of physiological race $a_1a_2A_3A_4$, isolate from Enkhuizen.

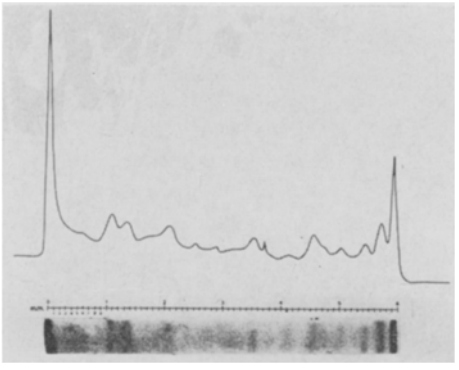

*Fig. 7. 25 µl monster met 0,094 mg eiwit van fysio $a_1a_2A_3A_4$, isolaat uit Enkhuizen.*

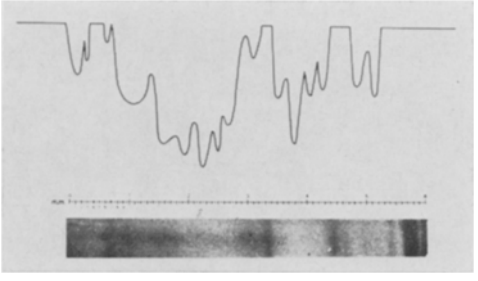

Fig. 8. 25 µl aliquot with 0.062 mg protein of physiological race $a_1a_2a_3A_4$, locality of isolation unknown.

*Fig. 8. 25 µl monster met 0,062 mg eiwit van fysio $a_1a_2a_3A_4$, plaats van onbekende herkomst.*

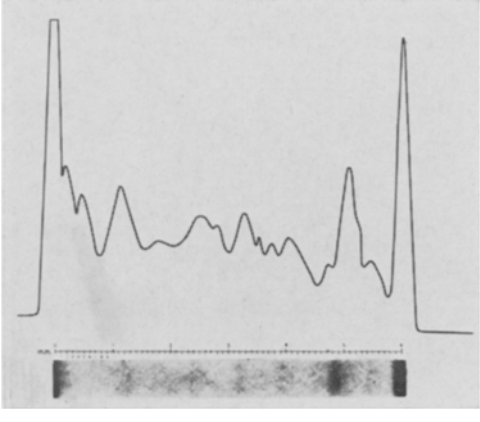

Fig. 9. 25 µl aliquot with 0.038 mg protein of physiological race $A_1a_2a_3a_4$, isolate from Bergschenhoek.

*Fig. 9. 25 µl monster met 0,038 mg eiwit van fysio $A_1a_2a_3a_4$, isolaat uit Bergschenboek.*

Fig. 10. Electropherograms of soluble extracellular proteins of 2 different isolates of *C. fulvum* with the same genotype for avirulence. A: 50 µl aliquot with 0.125 mg protein of physiological race $a_1a_2A_3a_4$, isolate from Drunen. B: 50 µl aliquot with 0.125 mg protein of the same physiological race, isolate from Wilnis.

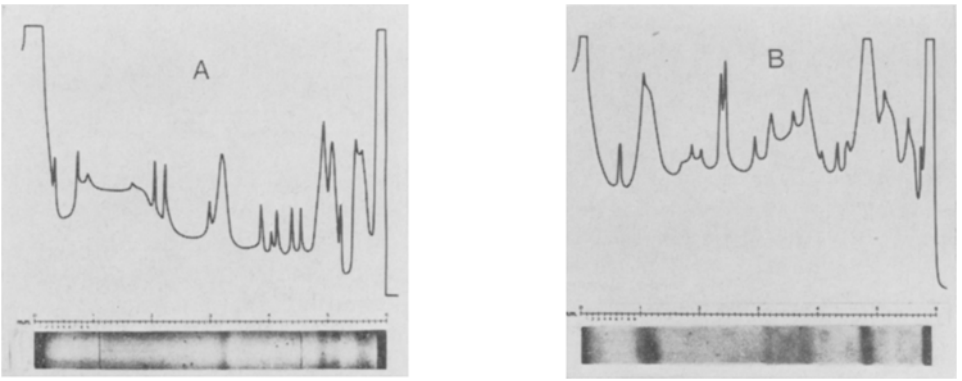


*Fig. 10. Elektroferogrammen van de oplosbare extracellulaire eiwitten van 2 verschillende isolaten van C. fulvum met hetzelfde genotype voor avirulentie: A. 50 µl monster met 0,125 mg eiwit van fysio $a_1a_2A_3a_4$, geïsoleerd in Drunen. B: 50 µl monster met 0,125 mg eiwit van hetzelfde fysio, geïsoleerd in Wilnis.*

Fig. 11. Electropherograms of soluble extracellular proteins of 2 different isolates of *C. fulvum* with the same genotype for avirulence. A: 25 µl aliquot with 0.038 mg protein of physiological race $A_1A_2A_3a_4$, isolate from Naaldwijk. B: 100 µl aliquot with 0.026 mg protein of the same physiological race, isolate from Den Hoorn.

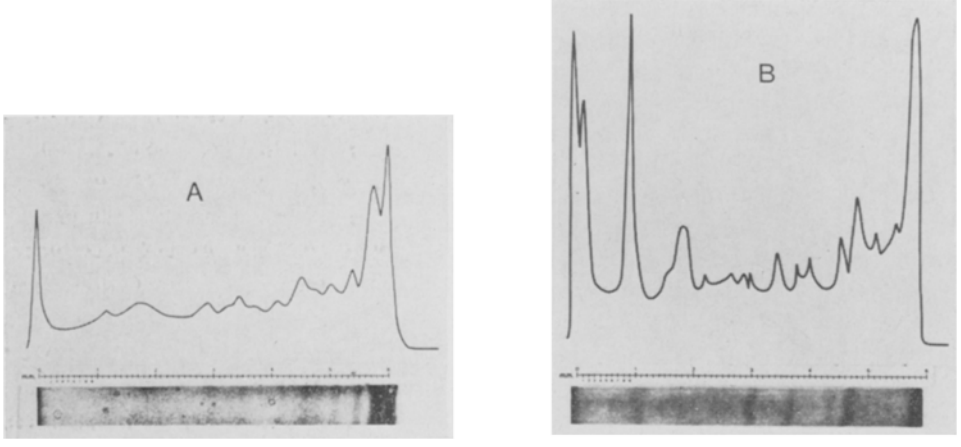


*Fig. 11. Elektroferogrammen van de oplosbare extracellulaire eiwitten van 2 verschillende isolaten van C. fulvum met hetzelfde genotype voor avirulentie. A: 25 µl monster met 0,038 mg eiwit van fysio $A_1A_2A_3a_4$, geïsoleerd in Naaldwijk. B: 100 µl monster met 0,026 mg eiwit van hetzelfde fysio, geïsoleerd in Den Hoorn.*

Electrophoresis of preparations from culture filtrates of a single isolate of *C. fulvum*, grown for different periods of time, showed always the same electrophoretical pattern. Therefore we could combine all preparations of one isolate to standardize our preparations.

Since the protein patterns obtained differed for each isolate, one must conclude that they are not characteristic for the physiological races.

## Discussion

Our data show clearly that the extracellular proteins of each isolate of *C. fulvum* examined have a different and reproducible pattern on disc gel electrophoresis; the pattern appears not specific for the physiological race to which the isolate belongs.

We have found earlier (van Dijkman, 1972; van Dijkman and Kaars Sijpesteijn, 1971, 1973) that the characteristic differences between the physiological races of *C. fulvum* are located in the extracellular high molecular weight fraction of culture filtrates of these organisms, races with a certain A-allele producing a specific compound which effects leakage in incompatible tomato host cells; this leakage is suggested to cause the hypersensitivity reaction. These A-allele-conditioned compounds may be proteins but no evidence has been obtained so far.

The protein patterns do not reveal any band characteristic for races with certain A-alleles, although by comparing the patterns of different isolates with the same set of avirulence alleles, one can exclude the bands present in neither gel (Fig. 10 and 11). This does not necessarily mean that the four compounds elaborated by the four A-alleles are no proteins, since they may be present in quantities insufficient to develop a visible band with Coomassie Brilliant Blue. Moreover, most of the proteins in the extracellular fraction will not be of any importance in the host-pathogen relation; also the solid bands may represent mixtures of several proteins.

The fact that the protein patterns differ for each isolate points to different mutations having taken place in the different isolates. No sexual processes are known of *C. fulvum* and this may limit the exchange of genetic material.

Our data agree with those of Macko et al. (1967) in as far as these authors found a difference in the soluble proteins of uredospores of two physiological races of *P. coronata*. Whether these differences are specific for the physiological races is, however, uncertain since the authors did not compare the protein patterns from different isolates having the same range of differential hosts.

Gill and Powell (1968) were unable to differentiate between physiological races of *P. fragariae* with this technique. The fact that we found consistent differences between the isolates of *C. fulvum* is probably due in part to the use of the soluble extracellular proteins from the culture filtrate, purified over Sephadex G-25, whereas most authors cited extracted the whole culture and used these protein preparations as such as sample for gel electrophoresis.

Electrophoretical patterns can be obtained rapidly and with relatively small amounts of material. Therefore, dis gel electrophoresis may be a tool for distinguishing isolates of *C. fulvum*.

**Acknowledgments**

The use of the Photovolt Gel Scanner of the Department of Virology, Agricultural University of Wageningen, was appreciated. We thank Mr J. Bours of the Institute for Anatomical Embryology, State University Utrecht, for using the Beckman Gilford Gel Scanner, Miss Gerda Verkuil for her very able technical assistance, Mr. J. Minnaard for making the drawings and the photographs, and Dr R. J. Lukens, Connecticut Agricultural Experiment Station, New Haven, Conn., USA, for carefully reading the English text.

**Samenvatting**

*Verschillen in disc gel elektroforesepatronen van oplosbare eiwitten uitgescheiden door verschillende fysio's en isolaten van Cladosporium fulvum.*

De extracellulaire oplosbare eiwitten van 15 verschillende isolaten, behorend tot 9 verschillende fysiologische rassen van *Cladosporium fulvum*, werden verkregen met behulp van Sephadex G-25 gelfiltratie van 3 tot 4-weken-oude filtraten van schudcultures. De drooggevroren fracties werden onderworpen aan elektroforese op polyacryl amide gels bij pH 9.5 en ongeveer 4 °C.

De met elektroforese verkregen eiwitpatronen bleken verschillend voor ieder isolaat onafhankelijk van het fysio waartoe zij behoren. Deze isolaten waren van verschillende plaatsen in Nederland afkomstig.

**References**

Bennick, A., 1968. Detection of persulfate in acrylamide gels. Analyt. Biochem. 26:453–457.

Chrambach, A., Reisfeld, R. A., Wijckoff, M. & Zaccari, J., 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. Analyt. Biochem. 20:150–154.

Clare, B. G., 1963. Starch-gel electrophoresis of proteins as an aid in identifying fungi. Nature, Lond. 200:803–804.

Davis, B. J., 1964. Disc electrophoresis II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121:404–427.

Durbin, R. D., 1966. Comparative gel electrophoretic investigation of the protein patterns of *Septoria* species. Nature, Lond. 210:1186–1187.

Dijkman, A. van, 1972. Natural resistance of tomato plants to *Cladosporium fulvum*. A biochemical study. Ph. D. Thesis, State University of Utrecht, the Netherlands.

Dijkman, A. van & Kaars Sijpesteijn, A., 1971. A biochemical mechanism for the gene-for-gene resistance of tomato to *Cladosporium fulvum*. Neth. J. Pl. Path. 77:14–24.

Dijkman, A. van & Kaars Sijpesteijn, A., 1973. Leakage of pre-absorbed $^{32}P$ from tomato leaf discs infiltrated with high molecular weight products of incompatible races of *Cladosporium fulvum*. Physiol. Pl. Path. 3: 57–67.

Gill, H. S. & Powell, D., 1963. Differentiation of three species of *Phytophthora* by polyacrylamide gel electrophoresis. Phytopathology 57:812.

Gill, H. S. & Powell, D., 1968. Polyacrylamide gel (disc) electrophoresis of physiologic races A 1 to A 8 of *Phytophthora fragariae*. Phytopathology 58:722–723.

Hall, R., Zentmyer, G. A. & Erwin, D. C., 1970. Approach to taxonomy of *Phytophthora* through acrylamide gel electrophoresis of proteins. Phytopathology 59:770–774.

Hubbeling, N., 1968. Attack of hitherto resistant tomato varieties by a new race of *Cladosporium fulvum* and resistance against it. Meded. Rijksfac. Landbouwwetensch. Gent 33:1011–1017.

Kooistra, E., 1964. Recent experiences of breeding leaf mould resistant tomatoes. Euphytica 13: 103–109.
Loening, O. E., 1967. The fractionation of high molecular weight RNA by polyacrylamide gel electrophoresis. Biochem. J. 102:251–257.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. J. biol. Chem. 193:265–275.
Macko, V. A., Novacky, A. & Stahmann, M. A., 1967. Protein patterns from uredospores of *Puccinia graminis* var. *tritici*. Phytopath. Z. 58:122–127.
Maurer, H. R., 1968. Disk-Electrophorese. W. de Gruyter & Co., Berlin (West-Germany), pp. 64–67.
Ornstein, L., 1964. The disc electrophoresis I. Background and theory. Ann. N.Y. Acad. Sci. 121: 321–349.
Shipton, W. A. & Fleischmann, G., 1969. Disc electrophoresis of proteins from uredospores of races of *Puccinia coronata* f.sp. *avenae*. Phytopathology 59:883.
Stipes, R. J., 1967. Disc electrophoresis of mycelial proteins from *Ceratocystis* species. Phytopathology 57:833.
Trudgill, D. L. & Carpenter, J. M., 1971. Disk electrophoresis of proteins of *Heterodera* species and pathotypes of *Heterodera rostochiensis*. Ann. appl. Biol. 69:35–41.

### Addresses

Organisch Chemisch Instituut TNO, Postbus 5009, Utrecht, the Netherlands.

## Book review

J. H. Franz & A. Krieg: Biologische Schädlingsbekämpfung. 208 pp. Verlag Paul Parey, Berlin und Hamburg, 1972. DM 24.–.

It was a pleasure to read this little book which deals with a subject that attracts so much attention at present. In a condensed form the authors discuss all major aspects of biological control. Not only the classical methods of biological control but also the newer ones that utilize physical or chemical stimuli of insect sense organs (light, sound, attractants, repellents, pheromones) as well as genetic control are dealt with. The book will be useful to those who need general information on biological control methods in their widest sense. It contains a number of photographs of the activity of parasites and predators which though good, do not contribute to the understanding of biological control problems. A rather extensive reference list is provided so that the student may read further into the subject.

G. W. Ankersmit