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ELECTROPHORETIC SEPARATION OF THE SOLUBLE PROTEINS OF *SCLEROTINIA SCLEROTIORUM, S. TRIFOLIORUM* AND *S. MINOR*

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R I N G K A S A N

Ekstrak protein dari Sclerotinia sclerotiorum, S. trifoliorum dan S. minor, sebagai jamur penyebab penyakit sayur-sayuran, telah dapat dipisahkan dengan cara "polyacrylamide gel electrophoresis". Berdasarkan pola-pola protein yang dibentuk, maka ketiga species tersebut dapat dibedakan, walaupun masih ada beberapa protein yang homolog yang dipunyai oleh ketiga species tersebut.

A B S T R A C T

Soluble proteins extracted from 3 species of Sclerotinia sclerotiorum, S. trifoliorum and S. minor were separated on polyacrylamide gel electrophoresis. The three species of Sclerotinia could be distinguished by their protein patterns.

INTRODUCTION

Recently gel electrophoresis of soluble proteins extracted from various fungal components has received considerable attention as a possible aid in resolving some of the problems inherent in fungal taxonomy.

Chang *et al.* (1962) found that different protein patterns obtained from *Neurospora sitophila, N. intermedia, N. crassa,*

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and a mutant strain of *N. crassa*, each had a distinctive protein complement and that interspecific differences were greater than intraspecific differences. Subsequently, Durbin (1966), working with species of *Septoria*, and Clare and Zentmeyer (1966) and Gill and Powell (1968), who studied species of the genus *Phytophthora*, all suggested that results obtained by using this technique would be useful as a taxonomic criterion. The electrophoretic patterns of the major proteins are characteristic of a species but no pattern characteristic of a genus has been recognized by Clare *et al.* (1968).

Macko *et al.* (1967) working with wheat stem rust, suggested that disc electrophoresis might be useful in characterizing subspecific taxa, i.e. formae specialis or physiologic races of wheat stem rust.

Whitney *et al.* (1968) working with *Verticillium albo-atrum*, *V. dahliae* and *Fusarium oxysporum*, found a large interspecific difference between *V. albo-atrum* and *V. dahliae* than the intergeneric difference between *V. dahliae* and *Fusarium oxysporum*. The same techniques had also been done by several authors (Shipton and Fleischmann, 1969; Glynn and Reid, 1969; Kulik and Brooks, 1970; and Milton *et al.*, 1971).

To elaborate this, changes in the electrophoretic patterns of proteins in the three species of *Sclerotinia* were undertaken in the present study.

MATERIALS AND METHODS

Isolates of *Sclerotinia sclerotiorum*, *S. trifoliorum* and *S. minor* used in this study were obtained from the culture collection of school of Botany, University of New South Wales, Australia. All the isolates were grown on Oxoids modified Czapek Dox Agar supplemented with 0.2% L-asparagine and 2 mg/l thiamine hydrochloride in Petri dish cultures. The cultures were incubated at 20°C under fluorescent light of about 15 lumens/sq.ft.

Sclerotia were collected when they had just become pigmented, usually between 2 and 3 weeks after inoculation. The sclerotia were extracted in a mortar containing a small amount of acid washed sand and a pestle, at 0° - 4°C. Three ml. of 0.05 M tris-glycine buffer (pH 8.7) were added to each gram of material. After thoroughly grinding, the mixture was centrifuged at 20.000 g for 1 hr. at 0° - 4°C. The resulting supernatant was decanted and dialyzed against distilled-deionized water for 24 hrs., then centrifuged for 90 min. at 20.000 g. The extracts were frozen rapidly using dry-ice and freeze-dried. The amount of protein in the resulting dry mass was determined by Folin-Ciocalteau method (Lowry *et al.*, 1951).

Gels were prepared by a modification of the method des-

cribed by Cheung and Marshall (1969).

Horizontal electrophoresis in 0.2 M tris-glycine buffer (pH 8.7) was performed at 4°C, with 32 mA d.c. until a tracker dye (bromophenol blue) had migrated 6 - 7 cm and this was usually after about 3 - 4 hours.

Each gel was sliced horizontally into four pieces of equal thickness and stained with Coomassie blue.

The protein bands were photographed against a diffuse white back ground illuminated from below. Also interpretative drawings were made from selected gels.

RESULTS

Considerable variation was found in the type of protein pattern obtained in extracts of the three species of *Sclerotinia* (Figure 1 and 2).

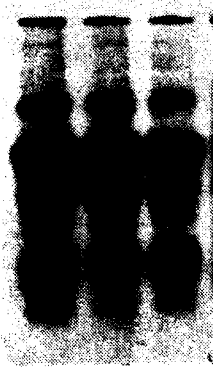

Figure 1

Electrophoretic spectrum on polyacrylamide gel of soluble proteins from *Sclerotinia sclerotiorum*.

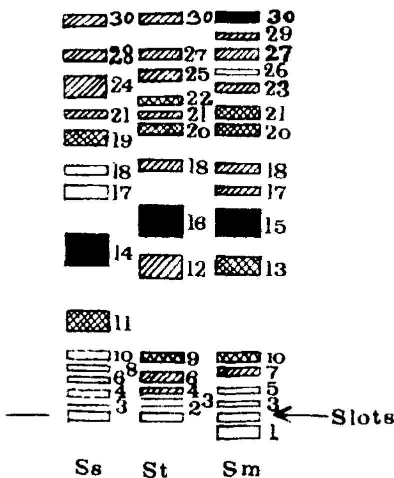

Figure 2

Diagrammatic representation of the electrophoretic spectra of the soluble proteins of *Sclerotinia sclerotiorum*, *S. trifoliorum* and *S. minor*. The intensities of the bands are represented by: black, very intense; cross-hatched, intense; single hatched, fairly intense; open, weak, stippled, resolution not in bands.

Ss, *Sclerotinia sclerotiorum*
St, *Sclerotinia trifoliorum*
Sm, *Sclerotinia minor*.

The electrophoretic protein patterns of all species observed here were similar, irrespective of their origin. Although those species produced essentially the same pattern, there were small variations within the different species in the number and position of electrophoretic bands.

Mean Ef values of bands from the three species of *Sclerotinia* are given in Table 1.

The staining intensities can be seen also in Figure 2. The extract of *S. sclerotiorum* and *S. trifoliorum* which very intense band is at number 7 and *S. minor* is number 6 from the slots, respectively.

Table 1

Mean Ef values of *Sclerotinia sclerotiorum*, *S. trifoliorum* and *S. minor*

No. of	Mean Ef values		
bands	*S. sclerotiorum*	*S. trifoliorum*	*S. minor*
1			- .02
2		.01	
3	.02	.02	.02
4	.04	.04	
5			.05
6	.07	.07	
7			.09
8	.10		
9		.11	
10	.12		.12
11	.20		
12		.31	
13			.32
14	.36		
15			.41
16		.42	
17	.48		.48

No. of	Mean Ef values		
bands	*S. sclerotiorum*	*S. trifoliorum*	*S. minor*
18	.53	.53	.53
19	.60		
20		.62	.62
21	.65	.65	.65
22		.67	
23			.70
24	.71		
25		.73	
26			.74
27		.77	.77
28	.78		
29			.82
30	.85	.85	.85

DISCUSSION

This investigation demonstrates that the electrophoretic patterns of proteins change both qualitatively and quantitatively in *Sclerotinia sclerotiorum, S. trifoliorum* and *S. minor*.

As stated in the introduction, the value of a new taxonomic method can only be assessed in relation to informations available from other disciplines.

The present results also indicate a larger interspecific difference between *S. sclerotiorum, S. trifoliorum* and *S. minor*.

As Clare, Flentje and Atkinson (1968) suggested, the patterns of buffer-soluble protein on gels following electrophoresis can be used to delimit fungal species.

The extent of variation shown in the electrophoretic patterns of proteins from cultures of *S. sclerotiorum, S. trifoliorum* and *S. minor,* might, however, be a peculiarity of the species of the genus *Sclerotinia,* which have capacity to change their morphological appearance and physiological behaviour is well known, in which case the protein patterns would reflect this diversity.

Polyacrylamide gel electrophoresis of the soluble proteins from each of the 3 species of *Sclerotinia* revealed a maximum of 14 protein fractions from *S. sclerotiorum*, 14 from *S. trifoliorum* and 16 from *S. minor* (Figure 2).

Investigators, who have studied proteins of other fungi with polyacrylamide gel electropgoresis reported fewer protein fractions. Chang *et al.* (1962) resolved 25 bands of *Neurospora* spp., Durbin (1966) reported 13 bands from 3 *Septoria* spp., Gill and Powell (1968) obtainde from 10 to 13 "components" from 3 species of *Phytophthora*.

Closeness of relationship between 2 taxa may be directly related to the number of homologous protein fractions that they posses in common. The number of homologous protein fractions among all possible paired combinations of the 3 species of *Scleortinia* used in this investigation are shown in Figure 2 and Table 1. "Homologous protein fractions" is meant as proteins from different taxa that migrate at the same distance from the origin. In making this comparison of each of the 3 species of *Sclerotinia* for homologous protein fractions, the data on the fractions obtained from each of the three species of *Sclerotinia*, were pooled to form a composite picture or spectrum of each species.

All taxa have common protein fractions with mean Ef values of .02, .53, .65 and .85. Two bands of *S. sclerotiorum* were common with *S. trifoliorum* (with mean Ef values of .04 and .07), another two bands of *S. sclerotiorum* were common with *S. minor* (with mean Ef values of .12 and .48). *S. trifoliorum* has two common bands with *S. minor* (with mean Ef values of .62 and .77). Each of them has independent bands, *S. sclerotiorum* has 6, *S. trifoliorum* has 6 and *S. minor* has 8 different protein fractions (Figure 2 and Table 1).

The results of the present investigation are based on the protein changes over the whole culture period and would seem to support the established taxonomy.

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