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A comparative study of solubilized proteins from phycomyces blakesleeannus by disc electrophoresis

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A COMPARATIVE STUDY OF SOLUBILIZED PROTEINS FROM *PHYCOMYCES BLAKESLEEANUS* BY DISC ELECTROPHORESIS

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by

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A COMPARATIVE STUDY OF SOLUBILIZED PROTEINS

FROM PHYCOMYCES BLAKESLEEANUS

BY DISC ELECTROPHORESIS

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ABSTRACT

Mycelial, sporangiophore, and zygospore solubilized protein fractions of *Phycomyces blakesleeanus* Burgeff were separated on polyacrylamide gels and stained. Protein patterns of 8 and 13 day old mycelia of the + strain were identical as were the patterns of the same two age groups of the - strain. The + mycelia exhibited 9 protein bands while the - strain contained 10 bands. Seven bands were homologous to both strains. Sporangiophore samples of 5 and 10 day old cultures from + and - strains contained 4 protein bands homologous to all 4 samples. The protein band at 9 mm was also homologous to all mycelial samples. The zygospore preparation yielded 2 protein bands. The band at 9 mm in this preparation was homologous with all mycelial and sporangiophore samples, while the band at 7 mm was unique to only the zygospore sample.

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INTRODUCTION

Recently, the use of polyacrylamide gel disc electrophoresis in fungal taxonomy studies has been reported (Chang, Srb, and Steward, 1962; Steward and Barber, 1964; and Stipes, 1970). Since Chang et al. (1962) indicated the superiority of disc electrophoresis of fungal proteins over other types of electrophoresis, great interest has been shown in this technique. Disc electrophoretic separation of proteins from different strains and mutants of Neurospora crassa was reported by Barber et al. (1969).

One objective of the present study was to define differences or similarities in mycelial protein patterns between the + and - strains of Phycomyces blakesleeanus of different ages using disc electrophoresis. There is no literature on electrophoretic separation of proteins from sporangiophores (the asexual stage) of any fungus. Another objective of this study was to compare protein patterns between + and - sporangiophores of different ages; and then secondarily to compare sporangiophore protein patterns with mycelial protein patterns. Since sporangiophore growth is initiated after 3 days of mycelial growth, protein patterns of 5 and 10 day old sporangiophores were compared with 8 and 13 day old mycelial protein patterns. Another objective of this study was to mate + and - mycelia of P. blakesleeanus

and subject the proteins from resulting zygospores to disc electrophoresis.

MATERIALS AND METHODS

Preparation of stock cultures

Cultures (+ and - strains) of the fungus Phycomyces blakesleeanus Burgeff on potato dextrose agar (PDA) were obtained from Carolina Biological Supply Company, Burlington, North Carolina. Subcultures of each strain were made on autoclaved (121 C, 15 min) PDA (Difco) in 10 ml screw cap test tubes. These new slants were refrigerated at 6 C and represented stock cultures.

Mycelial study

Two groups of mycelia of both strains were transferred from stock culture slants to petri dishes containing 15 ml of Bacto Sabouraud-maltose agar (Difco). Since P. blakesleeanus is thiamine deficient, (Burgeff 1934 and Schopfer 1934 in Bergman et al., 1969) thiamine (0.05 µg/ml of culture medium) was added to all culture media. The Sabouraud-maltose agar with thiamine was autoclaved at 121 C for 15 min.

The inoculated plates were wrapped in aluminum foil to insure only mycelial growth, and maintained at 23 C for 72 hr. One liter Erlenmeyer flasks were filled with 250 ml Bacto Sabouraud-maltose broth (Difco) with thiamine (0.05 µg/ml of broth) added. Flasks containing broth were plugged with cotton stoppers and autoclaved at 121 C for

15 min. The final pH was 5.6. A sterilized brass cork borer was used to make circular incisions of 1.0 cm diameter approximately 3.0 cm from the center of the agar plates. Six mycelial-agar discs from each plate were transferred to separate broth culture flasks. The inoculated flasks were lightly stoppered with cotton, wrapped in aluminum foil and maintained for 48 hr at 23 C. The first mycelial group (5 day old cultures) was then agitated at 60 rpm by a rotary shaker for 72 hr at 23 C. This process yielded 8 day old mycelia with no sporangiophores. The second mycelial group (5 day old cultures) was agitated for 8 days yielding 13 day old mycelia with no sporangiophores. The final pH of all culture broths was 4.8.

Mycelial samples were removed from the culture flasks, rinsed 3 times in distilled water in a Buchner funnel-suction flask apparatus, then homogenized in acetone (10 ml/g mycelia) at -10 C for 5 min in a Virtis 23 homogenizer to extract lipids. The entire homogenizing chamber was immersed in an acetone-dry ice bath to reduce protein denaturation. The homogenates were quickly suction filtered and rinsed 3 times in cold acetone. The final product in each case was a white powder which was refrigerated at -10 C until further use. A protein assay on each mycelial sample was determined by the Lowry method (Lowry et al., 1951).

Sporangiophore study

Mycelia from stock cultures of + and - strains were transferred to petri dishes of Sabouraud-maltose agar. The plates were maintained at 23 C in 12 hr alternating light and dark periods. After 72 hr of mycelial growth, sporangiophores appeared in both strains. Sporangiophores were removed with fine stainless steel forceps 5 and 10 days after initial growth. Sporangiophore samples were rinsed 3 times with distilled water and suction filtered. Each sample was homogenized in cold acetone (-10 C) in the Virtis 23 homogenizer. The homogenates were then rinsed and suction filtered in cold acetone. The final product in each case was a fine black powder which was refrigerated at -10 until further use. The soluble protein concentration of sporangiophore samples was determined by the Lowry method (Lowry et al., 1951).

Zygospore study

Mycelia of each strain were transferred from stock culture slants to opposite sides of Sabouraud-maltose agar petri dishes which were then wrapped in aluminum foil and maintained for 14 days at 23 C. Zygospores were plucked from the plates, rinsed with distilled water, homogenized in cold acetone and rinsed 3 times in cold acetone. The final product was a fine, dark powder which was refrigerated at -10 C until electrophoresis.

Gel preparation

The polyacrylamide gels were prepared by the method of Takayama et al. (1964). Glass tubes (5 mm ID x 85 mm long) were immersed in 1% dichlorodimethyl silane in benzene for 15 min. This silicon coat on the gel tubes facilitated the removal of the final gels. These tubes were then stoppered at their bases with special rubber stoppers, and the gel preparation was carefully pipetted into each tube to a height of 70 mm. The remaining portion of each tube was filled with 75% acetic acid. The tubes were then incubated at 50 C for 15 min for polymerization of the gels. After polymerization, the tubes were rinsed with 75% acetic acid and the remaining acid was removed from all gel columns. The final gel columns were placed in the electrophoretic unit which was that described by Davis (1964) and modified by Takayama et al. (1964).

Preparation of proteins for electrophoresis

Acetone powders from mycelia and sporangiophores were dissolved in a phenol-acetic acid-urea solution (16.7 : 8.3 : 3 v/v/w), pH 2.5, to a final protein concentration of 150 µg/ml. The zygospore sample was dissolved to a concentration of 0.005 g/ml in the above phenol solution. Bovine serum albumin (BSA) served as a standard for all electrophoretic runs and was pre-

pared by dissolving 0.005 g/10 ml of the above phenol solution.

Electrophoresis

One hundred microliters of each protein solution were applied to the gels. Both chambers of the electrophoretic apparatus were then filled with 10% acetic acid, pH 2.3. All gels were then electrophoresed at 5 mA/tube for 1½ hr. All mycelial samples were electrophoresed simultaneously as were the sporangiophore samples. Due to the extremely small amount of material recovered, only 1 gel containing the zygospore sample was run along with the mycelial samples. The gels were then carefully removed from the gel tubes and stained with 1% napthol blue black in 7% acetic acid for 1 hr. De-staining was accomplished with 7% acetic acid.

Gels were scanned by a Gilford Model 2410 Linear Transport with Adapter Model 2411 mounted to a Beckman DU Monochromator. The electrophoretographs were obtained at 570 mμ with a scan rate of 2 cm/min and a chart speed of 1 in/min, providing a record of absorbance vs. displacement. Electrophoretograph peak area readings were determined using a Gelman Model 39231 Compensating Polar Planimeter. A Linhof view camera with a Kodak red filter and Ektapan (ASA 100) sheet film were used for photographing the gels.

RESULTS

Standards

The bovine serum albumin standards included in each electrophoretic run yielded identical electrophoretic mobilities (Figure 1). The albumin in both standards migrated 23 mm from the origin at 5 mA for 1½ hr; hence, the remaining gels of the separate runs can be compared as to electrophoretic mobilities.

Mycelial study

The + strain of the 8 day old mycelia contained 9 distinct protein bands, while the - strain of the same age group contained 10 protein bands (Figure 2). Both strains contained 7 homologous protein bands at 9, 13, 16, 23, 29, 35, and 44 mm from the origin (Table 1). (Homologous protein bands refer to bands migrating the same distance from the origin). The + strain contained 2 protein bands at 5 and 37 mm which were not found in the - strain while the - strain contained 3 protein bands at 19, 26, and 51 mm which were not found in the + strain.

The + and - strains of 13 day old mycelia contained protein band patterns identical to their respective strains of 8 day old mycelia (Table 1, Figure 3). Planimetric readings of electrophoretographs indicated quantitative differences between the respective strains of the 2 age

groups (Table 1). The total area readings under the electrophoretograph peaks for the + and - strains of 8 day old mycelia were 3.52 and 3.16 sq in respectively, while the 13 day old mycelia total area readings were 2.56 and 2.14 sq in for the + and - strains respectively.

Sporangiophore study

The + strain of the 5 day old sporangiophores contained 4 protein bands, while the - strain of the same age group contained 6 bands (Figure 4). All 4 protein bands contained in the + strain were homologous to the first 4 protein bands of the - strain of the 5 day old sporangiophores (Table 2). However, the - strain contained 2 protein bands at 27 and 32 mm that were not found in the + strain.

Ten day old sporangiophores of both strains contained 4 protein bands that were not only homologous to their respective strains in the 5 day old group but also homologous to each other (Table 2, Figure 5). The protein band at 9 mm was homologous in all sporangiophore and mycelial samples. Quantitatively, the total areas under the protein band peaks of the + and - strains of the 5 day old sporangiophores were .14 and .69 sq in respectively, while the 10 day old sporangiophores' total area readings were .16 and .29 sq in for the + and - strains respectively.

Zygospore study

The solubilized proteins of the zygospores yielded a pattern of 2 faint bands at 7 and 9 mm respectively, containing approximately the same amount of protein (Table 3, Figure 6).

DISCUSSION

Mycelial study

The present study indicates the effectiveness of polyacrylamide gel disc electrophoresis in separating solubilized mycelial proteins of *Phycomyces blakesleeanus*. Not only are the protein patterns of the same protein solutions reproducible, but different protein solutions of respective strains of original stock cultures also yield the same results. Chang et al. (1962) indicated the superiority of disc electrophoretic separation of proteins of *Neurospora crassa* over other electrophoretic methods because of the greater number and better resolution of bands. Net charge and molecular size of proteins affect migration, thereby accounting for increased resolution (Ornstein, 1964).

As researchers have attempted to relate properties and structures of proteins to phylogeny (Anfinsen, 1959), disc electrophoresis has become widely employed in fungal taxonomy. Assuming that the relation between organisms is proportional to their genetic similarity, it is likely that the protein similarity between organisms would be a measure of their relationship (Sibley, 1964). Disc electrophoresis is helpful at specific and intraspecific levels of taxonomy where it is highly probable that proteins of equal mobility are structurally identical (Dessauer and

Fox, 1964).

The 8 and 13 day old mycelia of the + strain yielded identical protein patterns as did both age groups of the - strain. These data indicate apparent genetic stability in the respective strains with age. However, there is a difference in mycelial protein patterns between the + and - strains of Phycomyces blakesleeanus. Although 7 protein bands are homologous in both strains, 5 protein bands are not shared by the heterothallic strains (Table 1). The + strain contains 2 bands not found in the - strain, while the - strain contains 3 bands not found in the + strain. Since the 2 strains are of the same species and successfully mate to yield zygospores, they are undoubtedly genetically similar. Since equal amounts of solubilized proteins are represented in each gel, protein band pattern differences indicate a possible genetic difference between the 2 strains.

Quantitative differences that were observed were related to age rather than strain difference. In 6 of 9 bands of the + strain and 7 of 10 bands of the - strain, there was an increase in protein when the culture aged from 8 to 13 days (Table 1). The meaning of this increase in proteins is not known.

Sporangiophore study

Gels with sporangiophore proteins did not exhibit

the number of bands nor the magnitude of optical densities as did gels of mycelial proteins (Figures 4 and 5).

Four homologous protein bands appeared in all 4 sporangiophore samples (Table 2) with the 5 day old - strain possessing 2 additional bands. With these limited data, no conclusion is drawn concerning a qualitative difference between the strains of the 2 sporangiophore age groups. In addition, there does not appear to be a trend for protein concentration changes within homologous protein bands related to strain nor age of sporangiophores.

Since sporangiophore nuclei derive from mycelial nuclei (Bergman et al., 1969), the protein band at 9 mm occuring in all sporangiophore and mycelial samples apparently represents a functional genetic continuum between the two structures.

Zygospore study

Although very little zygospore material was recovered, 2 faint protein bands appeared on the gel (Figure 6). The protein band at 9 mm appeared also in all mycelial and sporangiophore preparations. However, the band at 7 mm is found only in the zygospores. This enhances the proposal that the + and - mycelial strains are genetically different.

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Table 1

Data from mycelial protein electrophoretographs.

8 day old, + strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.30	8.52	5
2	.75	21.30	9
3	.56	15.90	13
4	.84	23.85	16
5	.24	6.82	23
6	.25	7.10	29
7	.07	1.99	35
8	.34	9.66	37
9	.17	4.83	44
total = 3.52			

13 day old, + strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.36	14.10	5
2	.44	17.18	9
3	.43	16.78	13
4	.47	18.34	16
5	.21	8.21	23
6	.22	8.59	29
7	.11	4.30	35
8	.19	7.42	37
9	.13	5.08	44
total = 2.56			

8 day old, - strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.46	14.56	9
2	.69	21.82	13
3	.59	18.68	16
4	.48	15.19	19
5	.14	4.43	23
6	.08	2.53	26
7	.18	5.70	29
8	.22	6.96	35
9	.22	6.96	44
10	.10	3.16	51
total = 3.16			

13 day old, - strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.13	6.08	9
2	.40	18.69	13
3	.35	16.35	16
4	.35	16.35	19
5	.12	5.61	23
6	.12	5.61	26
7	.19	8.88	29
8	.21	9.81	35
9	.16	7.48	44
10	.11	5.14	51
total = 2.14			

Table 2

Data from sporangiophore protein electrophoretographs.

5 day old, + strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.04	28.58	9
2	.04	28.58	14
3	.04	28.58	18
4	.02	14.29	22
total = .14			

10 day old, + strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.02	12.50	9
2	.04	25.00	14
3	.05	31.24	18
4	.05	31.24	22
total = .16			

5 day old, - strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.20	29.00	9
2	.16	23.20	14
3	.09	13.04	18
4	.11	15.94	22
5	.07	10.13	27
6	.06	8.69	32
total = .69			

10 day old, - strain

band #	area (sq in)	% total area	distance from origin(mm)
1	.06	20.70	9
2	.09	31.00	14
3	.08	27.59	18
4	.06	20.70	22
total = .29			

Table 3

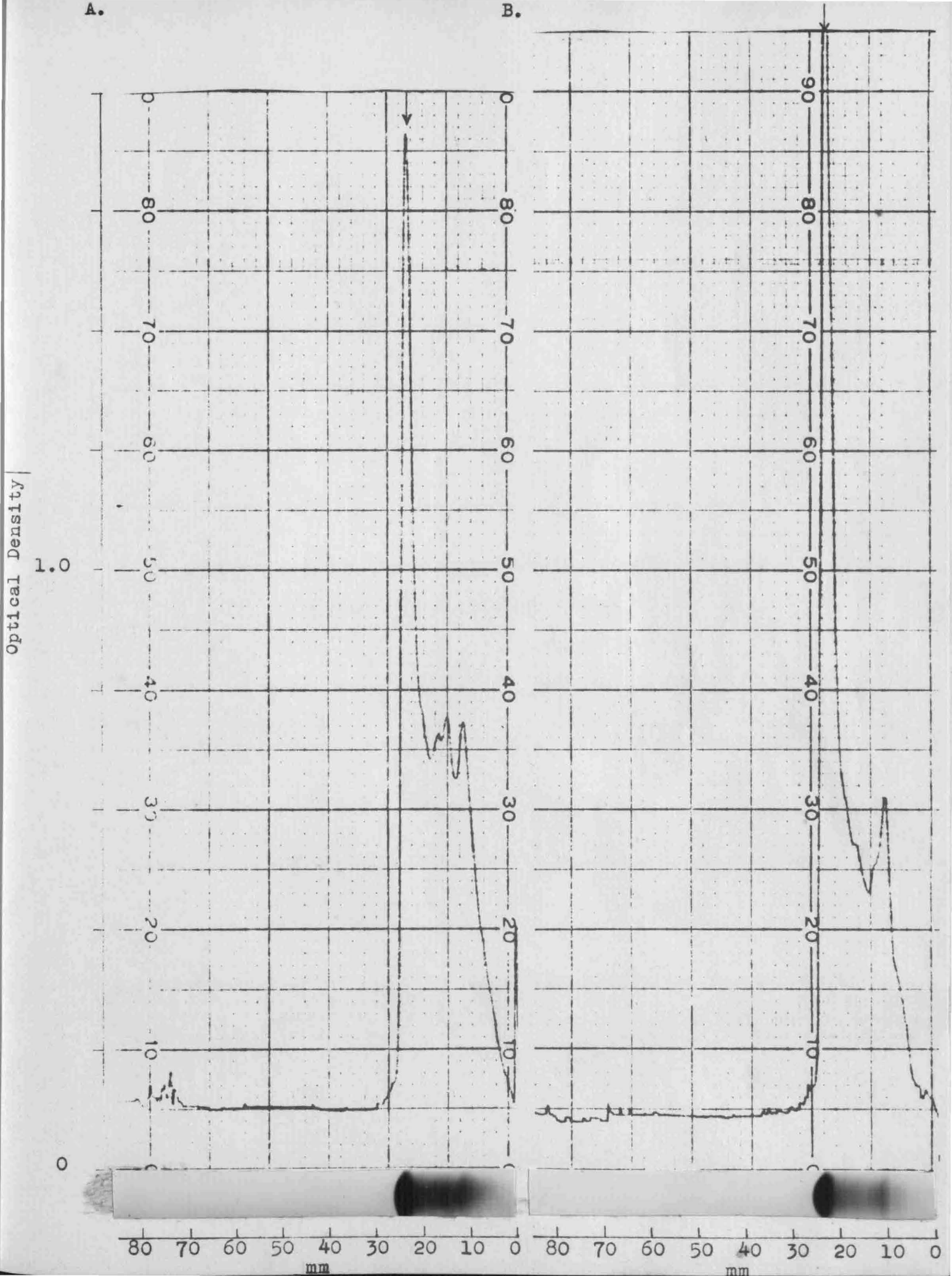
Data from zygospore protein electrophoretograph.

band #	area (sq in)	% total area	distance from origin(mm)
1	.04	50.00	7
2	.04	50.00	9
total =	.08		

Figure 1--Electrophoretographs and gel photographs of bovine serum albumin standards.

A--Standard from mycelial and zygospore electrophoretic run.

B--Standard from sporangiophore electrophoretic run.


A.
B.
Optical Density
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0
80 70 60 50 40 30 20 10 0
mm
80 70 60 50 40 30 20 10 0
mm

Figure 2--Electrophoretographs and gel photographs of 8 day old mycelial proteins.

A--(+) strain.

B--(-) strain.

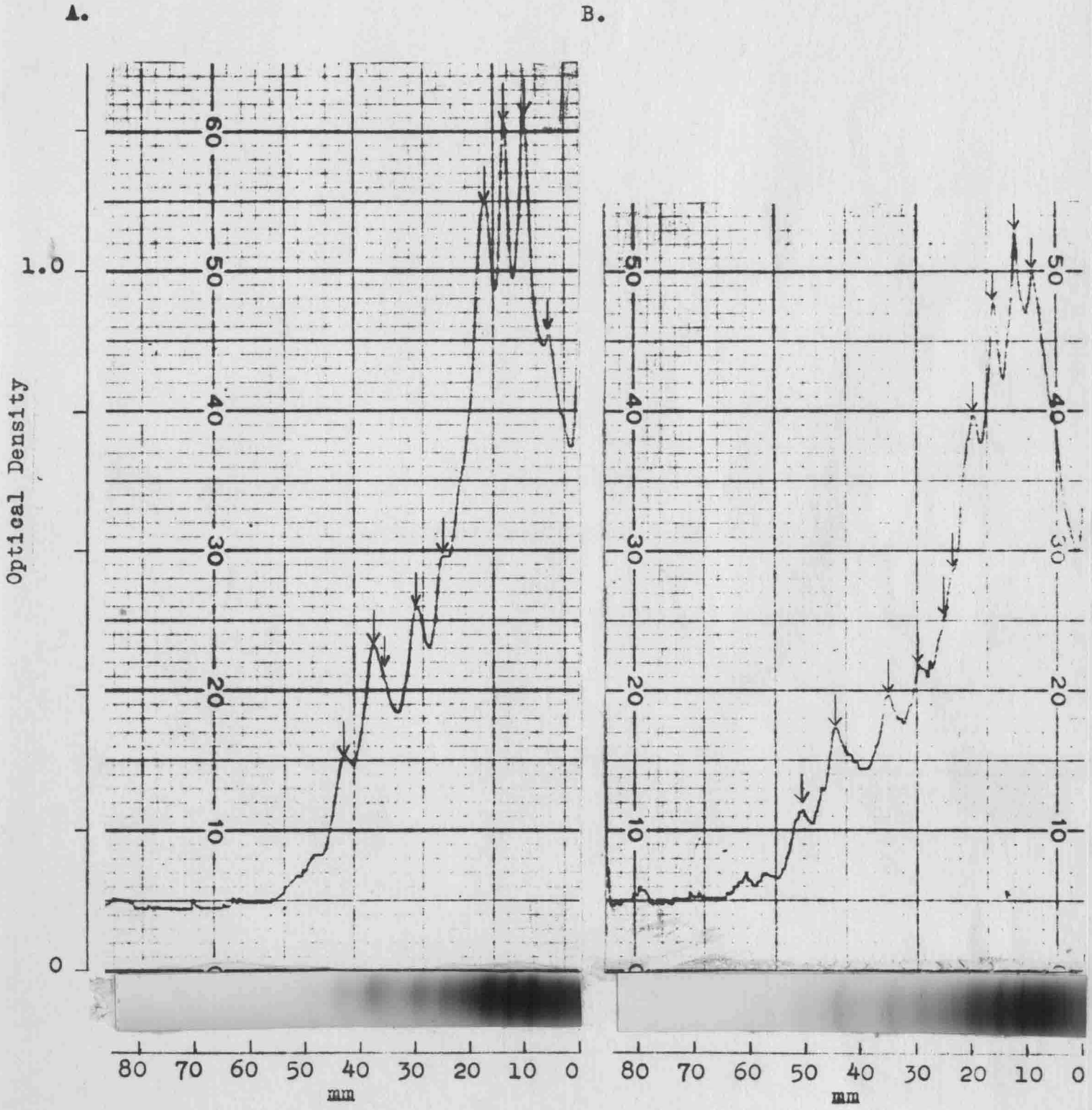
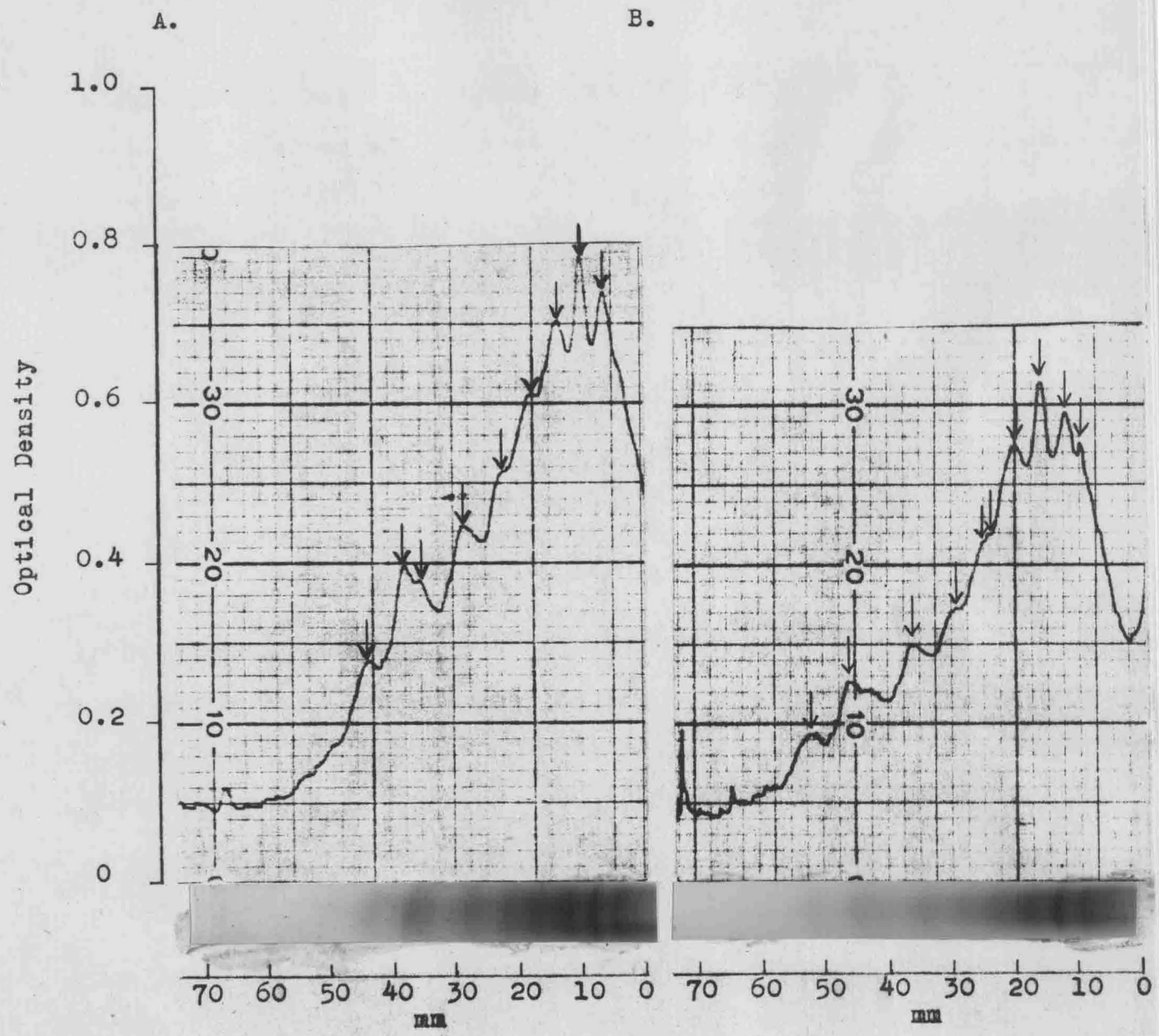

A.
B.
Optical Density
1.0
0
80 70 60 50 40 30 20 10 0
mm
80 70 60 50 40 30 20 10 0
mm

Figure 3--Electrophoretographs and gel photographs of 13 day old mycelial proteins.

A--(+) strain.

B--(-) strain.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B.
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0.2
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Optical Density
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20
10
70 60 50 40 30 20 10 0
70 60 50 40 30 20 10 0

Figure 4--Electrophoretographs and gel photographs of 5 day old sporangiophore proteins.

A--(+) strain.

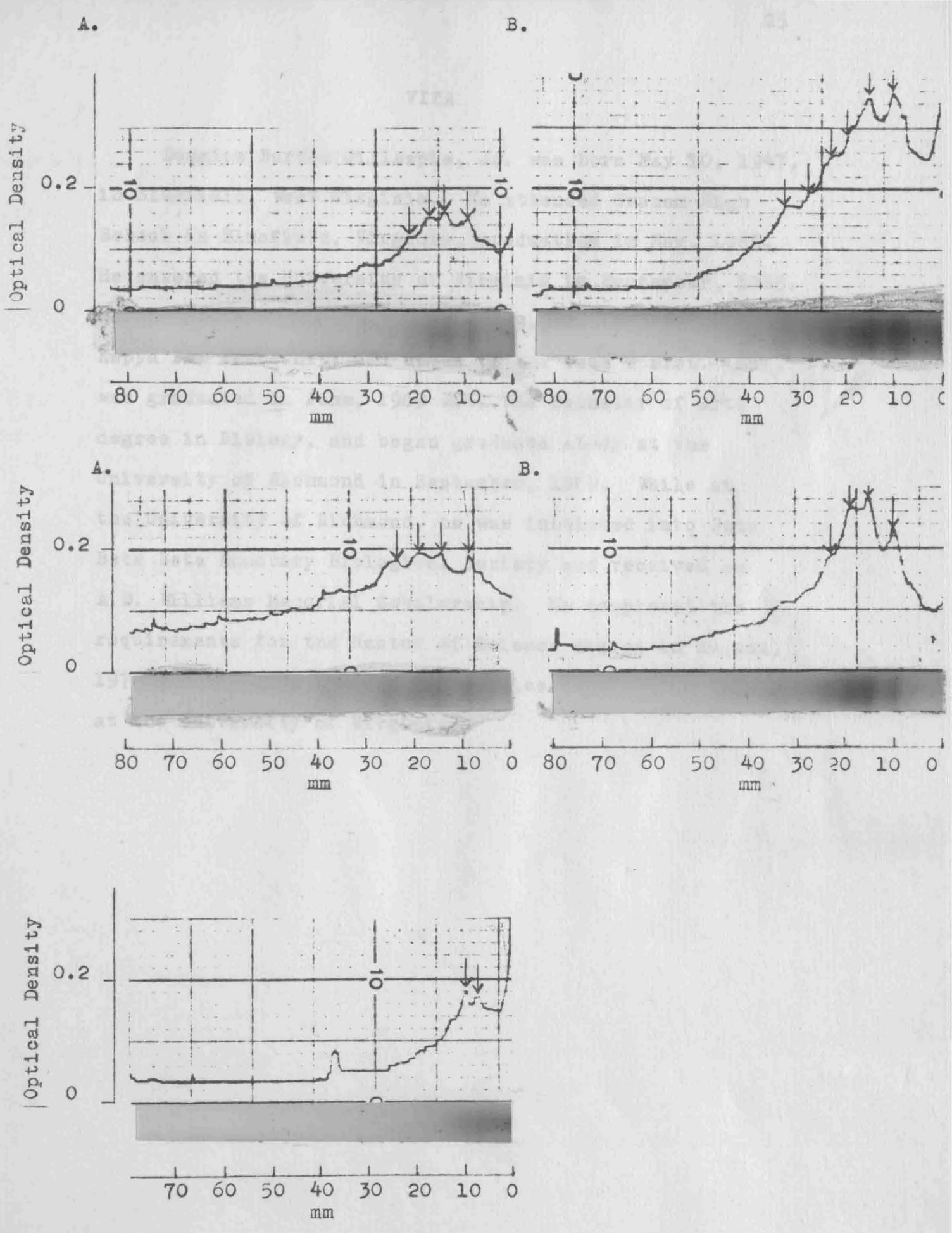
B--(-) strain.

Figure 5--Electrophoretographs and gel photographs of 10 day old sporangiophore proteins.

A--(+) strain.

B--(-) strain.

Figure 6--Electrophoretograph and gel photograph of zygospore proteins.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B.
Optical Density
0.2
0
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mm
A.
B.
Optical Density
0.2
0
80 70 60 50 40 30 20 10 0
mm
Optical Density
0.2
0
70 60 50 40 30 20 10 0
mm

VITA

Douglas Burton Gillespie, Jr. was born May 10, 1947, in Bluefield, West Virginia. He attended Graham High School in Bluefield, Virginia, graduating in May, 1965. He entered the University of Virginia in September, 1965. He was awarded a DuPont Scholarship, initiated into Phi Kappa Psi Fraternity and named to the Dean's List. He was graduated in June, 1969 with the Bachelor of Arts degree in Biology, and began graduate study at the University of Richmond in September, 1969. While at the University of Richmond, he was initiated into Beta Beta Beta Honorary Biological Society and received an A.D. Williams Memorial Scholarship. He completed the requirements for the Master of Science degree in August, 1971. He will pursue medical studies in September, 1971 at the University of Virginia.