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A DISC ELECTROPHORETIC STUDY OF PROTEINS OF BLUE-GREEN ALGAE

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Abstract—Proteins extracted from twenty-one cultures representing fourteen strains of blue-green algae were subjected to disc electrophoresis. Phycocyanin was visible on gels of all species and phycoerythrin on gels of seven species. All extracts gave substantially similar protein patterns although minor differences separate *Microcystis aeruginosa*, *Chlorogloea fritschii*, and especially *Anacystis nidulans*, both from each other and from the other species examined. A set of three protein bands was absent from cultures grown on a medium free of combined nitrogen, but present on similar cultures receiving such additions. However, the bands were also absent from two cultures grown with a combined nitrogen source when incubated at a relatively low light intensity.

INTRODUCTION

THE CLASSIFICATION of the blue-green algae poses many difficulties at all taxonomic levels. The most widely used system is that given in the flora of Geitler,[1] although some authors have adopted a rather different approach.[2] It has been argued[3] that a satisfactory classification will in fact be available only when there is enough data for a detailed numerical analysis. At present most of the information which could be used for such an analysis would come from classical morphology.

The potential value of disc electrophoretic studies of plant proteins for systematics has been emphasized,[4] and the relevance of data obtained by such studies to systems of classification of angiosperms has been demonstrated.[5] The high protein content of blue-green algae makes them suitable material for protein extraction and electrophoretic investigation. A further advantage accrues in that part of their protein is firmly attached to tetra-pyrrollic chains to form pigments which are soluble in dilute salt solution.[6] These considerations suggested that data useful for analysing the interrelationships of the blue-green algae might be obtained by a disc electrophoretic study of their extractable protein. Species were chosen for this study from a range of classical taxonomic groups. They include two organisms, *Anacystis nidulans* and *Chlorogloea fritschii* which, although much used in research, still cause argument as to their relationships.

[1] L. GEITLER, 'Cyanophyceae', in RABENHORST'S *Kryptogamen-Flora*. Akademische Verlagsgesellschaft m.b.H., Leipzig (1932).
[2] F. DROUET and W. A. DAILY, *Butler Univ. Bot. Studies* **12**, 1 (1956).
[3] B. A. WHITTON, *Brit. Phyc. Bull.* **3** (2), 412 (1967).
[4] D. BOULTER, D. A. THURMAN and B. L. TURNER, *Taxon* **15** (4), 135 (1966).
[5] D. BOULTER, D. A. THURMAN and E. DERBYSHIRE, *New Phytologist* **66**, 27 (1967).
[6] C. O hEOCHA, in *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN). Academic Press, New York (1965).

TABLE 1. SOURCE OF MATERIALS USED AND R_p VALUES OF PHYCOCYANIN AND PHYCOERYTHRIN AFTER DISC ELECTROPHORESIS OF PROTEINACEOUS EXTRACTS

Culture	Species	Source of algae	Durham culture No.	Whether axenic	Conditions of culture	Whether combined N in medium	R_p of pigment = bands: Phycocyanin Major	Phycocyanin Minor	Phycoerythrin Major	Phycoerythrin Minor
	Chroococcales									
1	*Microcystis aeruginosa*	Cambridge No. 1450/1	127	No	2000 lux, 35°, ASM−1	+N	0·46			
2	*Anacystis nidulans*	Westfield College, London No. 33	33	Yes	6000 lux, 35°, AC	+N	0·50	0 82		
3	*Anacystis nidulans*				1000 lux, 35°, AC	+N	0·50	0·82		
4	*Chlorogloea fritschii*	Westfield College, London No. 50	50A	Yes	6000 lux, 35°, AC	+N	0·46			
5	*Chlorogloea fritschii*		50B	Yes	6000 lux, 35°, AD	−N	0·44			
6	*Chlorogloea fritschii*		50C	Yes	Dark, 35°, AC+0·01 M sucrose	+N	Not visible			
7	*Chlorogloea fritschii*		50D	Yes	Dark, 35°, AD+0·01 M sucrose	−N	0·49			
	Hormogonales									
	(*a*) Rivulariaceae									
8	*Gloeotrichia echinulata*	Cambridge No. 1432/1	126	No	2500 lux, 20°, Chu 10	+N	+		+	
	(*b*) Microchaetaceae									
9	*Fremyella diplosiphon*	Cambridge No. 1429/1	135	Yes	6000 lux, 35°, AC	+N	0·49		0·40	

	(*c*) Scytonemataceae									
10	*Tolypothrix distorta* var. *symplocoides*	Field: University of Sierra Leone, wall of Botany Department		No	Partly shaded wall		0·52			
11	*T. distorta* var. *penicillata*	Field: Co. Durham Page Bank ponds		No	Floating mat on surface of pond		0·51		0·42	0·98
12	*Plectonema boryanum*	Cambridge No. 1462/4	106	No	2000 lux, 35°, AC	+N	?	0·73		0·95
	(*d*) Nostocaceae									
13	*Nostoc muscorum* 109	Cambridge No. 1453/9	109	Yes	6000 lux, 35°, AC	+N	0·50		0·37	0·85
14	*Nostoc muscorum* 109				2000 lux, 35°, AC	+N	0·50	0·71	0·38	
15	*N. muscorum* 110	Cambridge No. 1453/12	110	Yes	2000 lux, 35°, AC	+N	0·51	0·66	0·41	
16	*Anabaena variabilis*	Liverpool (N. Carr)	24B	Yes	2000 lux, 35°, AC	+N	0·49			
17	*A. flos-aquae*	Westfield College, London	125		6000 lux, 35°, Chu 10	+N	0·48	0·67		
18	*A. flos-aquae*				2000 lux, 35°, Chu 10	+N	0·46	0·69	0·37	
19	*Anabaenopsis* sp.	Westfield College, London No. 51	51A	Yes	6000 lux, 35°, AC	+N	0·51			
20	*Anabaenopsis* sp.		51B	Yes	6000 lux, 35°, AD	−N	0·50			
	(*e*) Oscillatoriaceae									
21	*Lyngbya* sp.	Indiana No. 621	114	Yes	2000 lux, 20°, AC	+N	0·45	0·73		0·92

RESULTS AND DISCUSSION

Pigments

In addition to the bromo-phenol blue indicator, pigments of two colours (red and blue) entered the small pore gel during electrophoresis. The blue pigment fluoresced magenta and the red pigment fluoresced orange in white light. They both migrated in the direction of the anode. At the end of the electrophoretic run, they each occupied a position on the gel which corresponded closely to that of a prominent protein band as shown by the Amido Black reaction. It was concluded that these pigments represented phycocyanobilin and phycoerythrobilin respectively still attached to their native proteins or to dissociated sub-units.[7]

With the sample loadings employed both pigments gave rise to well-defined bands presumably as the more dissociated forms. The major pigment bands were more diffuse and occasionally showed evidence of tailing. Nevertheless estimates of the positions of these bands agreed to within 0·03 R_p units[8] when similar samples were electrophorized on different occasions.

Because of this range of variation, differences between R_p values of the major pigment bands (Table 1) cannot be regarded as significant. Comparison of the relative positions of the more mobile blue pigment bands suggests that *Anacystis nidulans* may be distinct from the other species examined.

Phycoerythrin bands were seen on gels of extracts of seven of the twelve species examined. Although a typical phycoerythrin band was not observed on gels of *Chlorogloea fritschii*, electrophoresis of an extract of the culture of this alga grown in the dark with a source of combined nitrogen did result in gels on which there was a reproducible faint orange coloured band in a position corresponding to that of phycoerythrin in other species. It is noteworthy perhaps that these gels were the only ones which did not show a phycocyanin band.

"Total" Protein

Up to twenty bands were observed on any one gel after staining with Amido Black, and their distribution agreed with that reported for a variety of plant extracts. The position of any one band was reproducible on different occasions within the limits $\pm 0{\cdot}02$ R_p units. As in previous studies,[5, 8] the overall appearance of individual patterns was determined mainly by the relative position and intensity of the more intensely stained bands, particularly those with R_p values between 0·3 and 0·7.

The patterns formed on gels which had not shown red pigment bands were very similar to each other, and the position of the most prominent band corresponded to that of the phycocyanin on the unstained gel. This typical pattern is indicated in Fig. 1. The presence on these gels of a protein band with electrophoretic mobility similar to that of phycoerythrin cannot be assumed to indicate the formation of the protein of that pigment in all the species examined as it is possible for different proteins to migrate to the same position on a gel.[4, 9]

Despite the general similarity of the patterns, small reproducible differences were observed in some instances. The major band of *A. nidulans* moved faster, and that of *Microcystis aeruginose* slower, than the corresponding bands of the other species. The band R_p 0·40 was weaker on gels of *A. nidulans* in comparison with other gels. A band R_p 0·33 was prominent only on gels of extracts of *M. aeruginosa* and *C. fritschii*.

[7] D. S. BERNS, H. L. CRESPI and J. J. KATZ, *J. Am. Chem. Soc.* **85**, 8 (1963).
[8] D. J. FOX, D. A. THURMAN and D. BOULTER, *Phytochem.* **3**, 417 (1964).
[9] A. C. WILSON and N. O. KAPLAN, in *Taxonomic Biochemistry and Serology* (edited by C. A. LEONE), p. 321. Ronald Press, New York (1964).

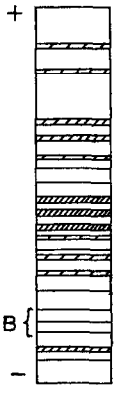

FIG. 1. PROTEIN PATTERN TYPICAL OF SPECIES OF BLUE-GREEN ALGAE.

The pattern illustrated was modified only slightly by the presence of phycoerythrin in extracts. Band R_p 0·40 was more prominent on gels of *Fremyella diplosiphon*, *Plectonema boryanum*, *Nostoc muscorum* (both strains), and *Lyngbya* sp., all of which showed phycoerythrin, than on gels which did not show this pigment. On gels of both strains of *N. muscorum* grown at 2000 lux illumination this band was even more prominent than the phycocyanin protein band.

Certain extracts (see below) gave gel patterns without the fine bands lettered B in Fig. 1. In the absence of these bands either an extra band was observed between the B region and the prominent band above it, or the prominent band was wider and possibly had fused with the extra band.

The set of bands lettered B in Fig. 1 was present on gels of extracts of an aged culture of *Anabaena flos-aquae* grown at 6000 lux illumination, but absent from gels of an extract of a young culture grown at 2000 lux. When extracts of *N. muscorum* strain 109 were electrophorized, the bands were again present on gels of the culture at 6000 lux, but absent from those of the culture at 2000 lux. Besides these two examples, the B bands were absent from the three cultures grown on a medium free of combined nitrogen, but present in similar cultures grown with nitrate present in the medium. The three examples are *C. fritschii* grown in the light and in the dark, and *Anabaenopsis* sp.

Heterotrophic cultures of *C. fritschii* gave gels on which at least one band was more intensely stained than on gels of autotrophic cultures of this species. For the culture free of combined nitrogen, the position of this band corresponded to that of the faint orange pigment referred to above: for the other culture the band had Rp 0·25.

Interpretation of the gel patterns of the two *Tolypothrix distorta* varieties was made difficult by background stain over the whole length of the gels. As far as could be judged both varieties gave the same pattern; the bands which could be discriminated from the background corresponded in relative intensity and spacing to bands of *N. muscorum*, but the proteins had not migrated as far as had those of that species.

A protein giving rise to a slow-moving band in the upper part of a gel might be a naturally occurring one, or it might be an artefact produced by association of smaller protein components; also the absence of a band from a gel might be due not to the absence of the corresponding protein from the extract, but to its presence in too low a concentration for detection

on the gel. For these reasons caution must be used in assessing the significance of differences based on the presence or absence of these bands.

The data presented indicate that among the organisms tested only minor differences occur in the patterns obtained by disc electrophoresis of their protein, and in general further use of the present technique seems likely to provide only a moderate amount of data useful in numerical analysis. However it does seem worth pointing out that *Anacystis nidulans*, frequently used in comparative research as an example of a blue–green alga, shows the greatest number of differences between its pattern and those of the other species tested. The fact that some differences do occur suggests that disc electrophoresis might well provide very interesting results if it were applied in a broader investigation to include forms like the Flexibacteria, *Rhodomicrobium* and the isolated chromatophores of small Cryptophyta.

EXPERIMENTAL

Materials

The sources of the algae used are indicated in Table 1, together with a summary of growth conditions. For several reasons the cultured organisms could not all be grown under the same standard conditions, and four different media were used: ASM-1,[10] Chu 10,[11] AC, AD. "AC" medium is based on the "C" medium of Kratz and Myers,[12] with the modifications that iron is added as Fe.EDTA chelate and the manganese content is reduced to one-tenth. "AD" medium is based on that described by Fogg,[13] with the modifications that iron was added to a concentration of 4 mg/l as Fe.EDTA chelate and that the trace element mixture used was that of Allen and Arnon.[14]

The cultures were harvested by centrifugation, freeze-dried and stored at −40°.

Extraction

Freeze-dried material (1 part) was ground in a mortar with acid-washed sand (1 part) and 1% (w/v) K_2SO_4 in 0·02 M phosphate buffer (pH 7·0) (3 parts) for 3 min; it was then decanted into glass centrifuge tubes and left to stand, with occasional stirring, for 30 min. The slurry was centrifuged at 1000 *g* for 5 min, the supernatant decanted into storage tubes and solid sucrose added to 25% (w/v).

Samples were electrophorized according to the method of Ornstein and Davis;[15] after measurement of the R_p values of the pigments the separated proteins were stained with Amido Black 10B.

The gel patterns characteristic of the pigment and of the stained proteins of extracts of *Anacystis nidulans* were established using as a control a globulin extract[5] of *Pisum sativum*. An extract of *A. nidulans* was included as control in every subsequent electrophoretic run.

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[10] P. R. GORHAM, J. MCLACHLAN, U. T. HAMMER and W. K. KIM, *Verhandl. Internat. Verein. Limnol.* **15**, 796 (1964).
[11] S. P. CHU, *J. Ecol.* **30**, 284 (1942).
[12] W. A. KRATZ and J. MYERS, *Am. J. Botany* **42**, 282 (1955).
[13] G. E. FOGG, *Ann. Botany*, **13**, 241 (1949).
[14] M. B. ALLEN and D I. ARNON, *Plant Physiol.* **30**, 366 (1955).
[15] L. ORNSTEIN and B. J. DAVIS, *Disc Electrophoresis.* Preprint by Distillation Products Industries (Eastern Kodak Co.), Rochester, New York (1961).