OBSERVATION ON THE FREQUENCY OF HETEROCYSTS AND ROLE OF HETEROCYSTS IN SPORULATION IN ANABAENA (CYANOPHYCEAE)

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Abstract

The relationship of photosynthesis to light intensity in natural algal populations is of considerable theoretical interest. Most of the works concern with chemical composition of the media. This investigation is the result of the study of the effect of intensity and quality of light and various nutrient media on frequency of heterocyst formation and role of heterocysts in sporulation in three species of *Anabaena cylindrica* Lemm: *A. hallensis* (Janz.) Born. et Flah, *A. variabilis* Kutz.

Introduction

The filamentous members of heterocystous blue green algae Anabaena during vegetative growth, the formation of spores appears at a specific position in relation to heterocyst. The process of spore formation in which the vegetative cell differentiate into spore varies in various members of Cyanophyceae.

Carter (1856) was the first to suggest that heterocyst may function in the control of sporulation in blue green algae. This hypothesis was supported by Fritsch (1904, 1951), Geitler (1921), Demeter (1956), Wolk (1966) and Singh & Sirvastava (1968).

In *Cylinderospermum* Glade (1914) and *Gloeotrichia* Singh & Tiwari (1970) conspicuous spores differentiate into spores in the immediate vicinity of terminal heterocyst. In Anabaena the process of sporulation varies with species. In *A. cylindrica* the process is sequential, i.e., beginning adjacent to the heterocyst and

proceeding away from it, suggesting that heterocysts play role in sporulation (Wolk, 1966). This hypothesis gets support from observations of Fritsch (1951), that heterocysts secret certain substance that stimulate akinete formation. On the other hand Singh and Sirvastava (1968) attribute sporulation in *A. dellolum* to concentration of nitrogen in the medium. This observation is in agreement to the findings of Glade (1914), Harder (1917), Canabaeus (1929) and Demeter (1956). According to these authors spore formation depends or varies with nitrogen and phosphate concentrations in the culture media Glade (1914); spore formation depends upon nitrate concentratidn Harder (1917), Nitrogen and phosphate concentration plays a definite role in sporulation in five species of *Anabaena* Canabaeus (1929) Nitrogen has an stimulating action on spore formation Demeter (1956). Sporulation depends upon many factors besides media, it depends upon characters of inoculum and temperature Wolk (1965). In *A. doliolum* Singh & Shrivastava (1968) sporulation begins away from heterocyst beginning midway between heterocyst and proceeding centrifugally, indicating that heterocyst play an inhibitory role Talpasayi and Bahal, (1967).

Material and Methods

The experimental cultures of the three species of Anabaena, i.e., *A. cylindrica*, *A. hallensis* and *A. variabilis* were raised from spores on media De. The pattern of spore germination, time period of appearance of first heterocyst after inoculation, position of first heterocyst, frequency of first heterocyst after insulation, percentage of heterocysts in respect of vegetative cells, relative position of heterocysts and spores and intensity of spore formation were observed microscopically and in–camera. Studies were also conducted to observe the life cycle, i.e., from spore germination to spore formation. Frequency of heterocyst was calculated by the method suggested by Fogg (1951). Studies in–camera were made by mounting rectangular pieces of agar blocks containing spores, on microscope slides and were sealed with wax. Care was taken that these blocks contained enough amount of moisture and oxygen. This was done by cutting the block into 3 pieces leaving some space between them. The effect of light (intensity and quality) and influence of culture media on the rate of heterocyst formation and sporulation was observed by subjecting these cultures to various intensities of light (1000, 2000, 3000, 4000 and 5000 Lux, white fluorescent light). For quality, red and blue light (1000 Lux) was used. Influence of culture media was studied by raising cultures on media suggested by Allen and Arnon (1955) (illuminated under white fluorescent light at an intensity of 1000 Lux). All these experiments were conducted under continuous light.

Discussion

The heterocyst percentage was determined following Fogg (1951). It was found that the rate of heterocyst formation varies differently, not only under different conditions, but also at different periods of growth (Table 1) In *A. cylindrica* after 4th day of culturing, the heterocysts were found to be in lesser degree, while *A. hellensis,* an increase in the intensity of light displayed an increase in heterocyst percentage.

It can be seen that one and the same species, subjected to external conditions, the percentage of heterocysts under the influence of external factors either remains more or less constant along the period of growth or it shows variation. Besides that, under one and same culture conditions the species shows a marked variation in heterocyst percentage.

In case of *A hallensis* it was observed that in conditions where percentage of heterocysts is low (white light. media De) not only increase in spore formation appeared but initiation of sporulation was also earlier, as compared to other species. The sporulation was distal to heterocyst i.e., beginning midway between two heterocysts (Fig.1). This phenomenon clearly proves that heterocysts do not have influence on sporulation. Our findings also support the hypothesis put forward by Singh and Sirvastava (1968).

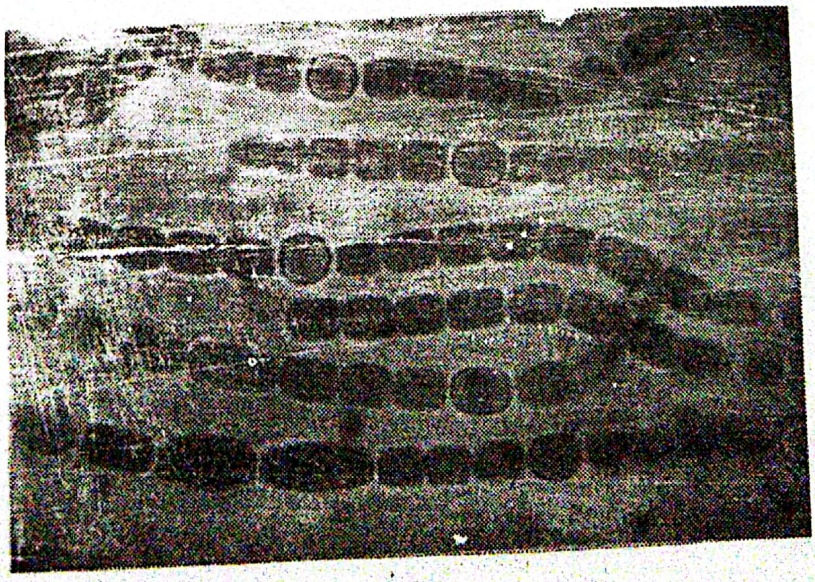

Fig.1

Table 1.

HETEROCYST PERCENTAGE (9%) IN SPECIES OF ANABAENA

Culture conditions Light Quality Colour	Intensity in Luxs	Media	4th day A. cylindrica	A. hallensis	A. variabilis	8th day A. cylindrica	A. hallensis	A. variabilis
White	1000	De	47.42	8.27	5.97	9.61	4.45	7.26
Red	1000	De	6.45	9.03	7.44	9.68	12.44	9.15
Blue	1000	De	5.20	0.95	5.80	5.45	6.53	5.F
White	2000	De	9.89	9.62	9.43	11.52	S.F	9.66
White	3000	De	10.70	7.91	9.43	12.22	S.F	9.40
White	4000	De	12.50	6.42	8.46	13.68	S.F	10.31
White	5000	De	13.59	4.88	7.54	14.87	S.F	10.74
White	1000	Allen	10.78	15.06	10.04	4.36	10.39	11.66

	12th day A. cylindrica	A. hallensis	A. variabilis	16th day A. cylindrica	A. hallensis	A. variabilis
	10.10	16.95	7.53	12.21	S.F	6.5
	9.68	10.00	12.60	12.60	S.F	9.32
	7.67	15.99	S.F	9.92	S.F	S.F
	12.28	S.F	9.33	13.85	S.F	3.10
	14.22	S.F	9.45	130.30	S.F	3.10
	13.34	S.F	S.F	12.80	S.F	S.F
	13.00	S.F	S.F	12.74	S.F	S.F
	10.34	S.F	6.91	S.F	S.F	S.F

S.F. = Sporo Formation

In *A. variabilis* culture conditions stimulating heterocyst and spore formation do not coincide with. It can be noted (Table 1) that in cultures that were placed in white light at an intensity of 1000 Lux media De (1939) sporulation did not occurs. in spite of the good percentage of heterocyst, while under the influence of blue light of 1000 Lux intensity media De (1939) sporulation was present even at low percentage of heterocysts.

In *A. variabilis* the mode of sporulation was neither proximal nor distal in true sense, i.e., no constant pattern of sporulation was recorded. Initiation of sporulation started in any cell between two heterocysts. In *A. cylindrica* sporulation was of proximal nature. Based on experimental evidence, in respect of role of heterocysts in sporulation our observations are quite in-agreement with that of Singh and Srivatstva (1968). The role of heterocysts in sporulation is as yet incompletely understood and in our opinion before arriving at any definite conclusion this needs further investigation.

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