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ENVIRONMENTAL FACTORS AFFECTING THE PLAQUE FORMING ABILITY OF BLUE-GREEN ALGAE VIRUS LPP-1

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ABSTRACT

The ability of the blue-green algal virus to develop plaques under different intensities of Gro-lux and fluroescent lighting at 19 ± 2°C was assessed. The algae for virus plaques were grown according to the soft agar overlay technique. Data indicated that in Gro-lux, the plaque size and the number increased as the light intensity increased from 125 ft-c to 400 ft-c. However, no such correlation was observed with fluorescent light.

INTRODUCTION

The susceptibility of blue-green algae to virus diseases was established by Safferman and Morris in 1963, with the isolation of blue-green algae virus (BGAV). The virus was isolated from Indiana waste stabilization ponds which initially lysed the filamentous blue-green algae Plectonema boryanum. The virus host range has been shown to include several other species of Plectonema as well as members of the other genera, Phormidium and Lyngbya (Safferman and Morris, 1964a). Upon isolation of this virus, it was recognized by Safferman and Morris in 1964b that viruses of this type could have wide spread application in the control of troublesome algal growth. Singh and Singh (1967) isolated five strains of cyanophages, two of which differed in host range and plaque morphology, while the remaining three brought about lysates of LPP-1 host Plectonema boryanum although showing variation in growth behavior and plaque size. Safferman and Morris (1964a) isolated two distinct plaque types from the virus LPP-1 on the basis of their size. The LPP-1 virus is more closely allied to bacteriophages than to viruses of higher plants (Schneider et al 1964; Smith et al 1966a; and Smith et al 1966b). Goldstein (1967) isolated the DNA and showed that it was doubly stranded with a molecular weight of $51 \times 10^6 \pm 3 \times 10^6$ daltons. Smith et al (1967) and Brown et al (1966) made a comprehensive study of the growth cycle of the LPP-1 virus. The present research was conducted to assess the effects of physiological conditions on the infection process and on the plaque-forming ability of BGAV.

MATERIALS AND METHODS

A large plaque former, blue-green algal virus strain LPP-1 (conc. 2 x 10^8 plaque forming unit/ml) was received from Safferman (Federal Water Pollution Control Administration, U.S. Department of the Interior, Cincinnati, Ohio). Three week old culture of Plectonema boryanum (P594) lysed in 5 days after inoculation. The lysed algal culture was centrifuged at 10,000 rpm in a refrigerated RC2-B centrifuge for 10 min and the supernatant fluid was passed through a millipore filter (GS 0.22 u, white plain, 47 mm size). This partially purified virus was stored at 4°C and was used in all the experimental work.

Dilutions of the virus were made with the salt solution containing 0.2 g $MgCl_2 \cdot 6H_2O$, 5.85 g NaCl, and distilled water to bring the volume to one liter. 10^{-5} virus dilution was used for Plectonema boryanum (strain P581, P594 and P597), and 10^{-2} virus dilution was used for Lyngbya species (strain L487 and L488).

PREPARATION OF PETRI PLATES FOR VIRUS ASSAY. Fifteen grams of agar in a liter of Bold's basal medium (BBM) was autoclaved for 15 min at 15 pounds pressure. Forty ml of this preparation was poured into each Petri plate and allowed to solidify at 25°C. The surface layer for each strain was prepared in a 50 ml sterilized beaker consisting of 2.0 ml of an appropriately diluted virus (10^{-5} for P581, P594, P597 and 10^{-2} for L488 and L487), 8 ml of 3 to 4-week old algae, and 10 ml of 0.5% nutrient agar which had been previously autoclaved and cooled to 47°C. Out of this 20 ml mixture, 5 ml was poured into each of four Petri plates. The entire procedure was carried out in an ultraviolet transfer room. The Petri plates inoculated as above were incubated in a specially made growth chamber which was equipped with 3 light filters and several temperature control ventilators. This growth chamber was housed in a 7°C cold room. The temperature inside the growth chamber was controlled through the ventilators, and the light intensity was maintained through removable filters. The quality of light was provided by using Gro-lux and flourescent light tubes, and the illumination was continuous. The temperature was maintained consistently at 19° ± 2°C. The number of plaques were counted after three days of incubation by marking the plate into several compartments. The size of randomly selected plaques in a plate were measured from edge to edge with a K 16x Zeiss micrometer. Two experiments were conducted per light intensity with four replications in each strain. An average of the two experiments was taken. The size of the plaque is given in millimeters.

RESULTS AND DISCUSSION

FLUORESCENT LIGHT AND BGAV PLAQUES. When plague-forming ability of the BGAV was tested at 200 ft-c light intensity, the highest number of plaques obtained were in P594 as compared to all other strains tested. P597 was the least susceptible to the virus as indicated by the number of plaques developed (Table 1).

Among Lyngbya species, L488 was more susceptible to virus than L487. L488 was less susceptible than P594. It is apparent from Table 1 that plaque size did not correlate with plaque number. However, plaques were larger with P581 and L488 than the other strains, and smallest with P597.

Under 130 ft-c light intensity, P594 produced the highest number of plaques than other strains in both Plectonema boryanum and Lyngbya species. The size of the plaques were almost similar in all the strains, although slightly larger plaques were developed in P581 as compared to other strains. In general, under 130 ft-c light intensity, plaques developed were eight times more in number and three times smaller than when both the species were grown under 200 ft-c light intensity. Under 500 ft-c light intensity, strain L488 developed more and larger plaques than all other strains in both Plectonema boryanum and Lyngbya species (Table 1). Seven hundred ft-c was the highest intensity used in the fluorescent spectrum. There is a great variation in the number of plaques developed in various strains of Plectonema boryanum and Lyngbya species when incubated under 700 ft-c light intensity. The lowest number of plaques appeared in P597. The size of the plaques was largest in P581 (3.45 mm) and the smallest in L487 (1.09 mm). Therefore, it could be concluded from this data that the light intensities used did not show correlation between size and the number of plaques. However, it seemed that at 200 ft-c light intensity there was correlation between the low number of plaques and larger size of lesions.

GRO-LUX LIGHT AND BGAV PLAQUES. The behavior of Plectonema boryanum and Lyngbya species in regard to plaque number and size varies with the different strains at 125 ft-c Gro-lux light. Strains L488 produced the highest number of plaques and the largest size as compared to other strains tested. Strain L487 gave rise to the lowest number of plaques of fairly large size (1.89 mm). There seemed to be little correlation between the plaque size and number (Table 2).

The number of plaques formed under 300 ft-c Gro-lux light were highest in number and of largest size in P597 as compared to all other strains. On the contrary, strains L487 produced the least number and the smallest of plaques (Table 2). Good correlation between plaque size and number was evident with P597 and L487. Plectonema boryanum and Lyngbya species produced uniformly the highest number of plaques of largest size with 400 ft-c Gro-lux light intensity. Strain P597 developed the highest number and P581 developed the largest size plaques (Table 2).

In general, it can be concluded that as the Gro-lux light intensity increased from 125 ft-c to 400 ft-c the plaque size and number increased. No such correlation could be deducted in fluorescent light. In comparing fluorescent light with Gro-lux, it seemed that the latter produced more plaques and of larger size in both Plectonema boryanum and Lyngbya species.

TABLE 1: Effect of fluorescent light intensities at 19 ± 2°C on the size and number of blue-green algae virus plaques developed on Plectonema boryanum and Lyngbya species.

Algae	Light Intensity							
	130 ft-c		200 ft-c		500 ft-c		700 ft-c	
	No. of Plaques	Size of Plaques (mm)	No. of Plaques	Size of Plaques (mm)	No. of Plaques	Size of Plaques (mm)	No. of Plaques	Size of Plaques (mm)
Plectonema								
Strains								
P581	151	2.53	21	6.74	150	1.80	25	3.45
P594	301	2.16	30	5.49	170	1.79	200	2.07
P597	285	2.02	10	1.29	167	1.87	10	2.27
Lyngbya								
Strains								
L487	116	2.18	28	4.42	89	1.64	89	1.09
L488	229	2.47	15	6.27	212	2.03	238	2.91

TABLE 2: Effect of Gro-lux light intensities at 19 ± 2°C on the size and number of blue-green algae virus plaques developed on Plectonema boryanum and Lyngbya species.

Algae	Light Intensity					
	125 ft-c		300 ft-c		400 ft-c	
	No. of Plaques	Size of Plaques (mm)	No. of Plaques	Size of Plaques (mm)	No. of Plaques	Size of Plaques (mm)
Plectonema						
Strains						
P581	117	1.71	150	1.94	202	3.62
P594	76	1.87	161	1.55	319	2.56
P597	146	2.65	317	3.52	218	2.67
Lyngbya						
Strains						
L487	38	2.60	93	0.26	256	2.61
L488	162	3.18	136	1.34	216	2.62

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