

EFFECT OF THE ALGAE *Chlorella vulgaris* AND *Scenedesmus obliquus*  
ON SURVIVAL OF BACTERIOPHAGES OF *Escherichia coli*

(UDC 576.858.9:576.851.48.095.877:582.264)

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Translated from *Byulleten' Èksperimental'noi Biologii i Meditsiny*, Vol. 61, No. 6,  
pp. 71-74, June, 1966

Original article submitted September 10, 1964

As mentioned earlier [1, 2], algae differ in their effect on bacteriophages. If the action is one of inhibition, the conditions are created for intensive proliferation of bacteria. This is particularly important in connection with the interaction between bacteriophages and *Escherichia coli*, an organism used to indicate the hygienic state of reservoirs.

The object of the present investigation was to study the effect of plankton algae on the bacteriophages of *E. coli* in different concentrations.

#### EXPERIMENTAL METHOD

Experiments were carried out with algologically and bacteriologically pure cultures of green protococcal algae *Chlorella vulgaris* and *Scenedesmus obliquus*, and three strains of bacteriophages specific for *E. coli*: virulent ( $T_1$ ,  $T_4$ ) and moderately virulent ( $\lambda$ ). The cultures of the phages and the reference strain of *E. coli* were obtained from the laboratory of Genetics of Microorganisms, Institute of Experimental Biology, USSR Academy of Medical Sciences.\* The phage proliferated on the reference strain of *E. coli* commune in meat-peptone broth.

The biological activity of the bacteriophages was determined from the number of free corpuscles capable of giving negative colonies on a solid medium seeded with the reference strain of *E. coli*. Seedings were made on meat-peptone agar by the double-layer method. Counts of the cells of the algae in the ML-2 luminescence microscope showed that isolated dead cells appeared in the culture on the 10th-12th day of development, and by the 15th day their number had reached 0.1-0.2% of the initial concentration. The yield of algae was expressed as the number of cells per ml. The remainder of the techniques were similar to those described earlier [2, 3].

#### EXPERIMENTAL RESULTS

Preliminary experiments showed that the bacteriophages survived in Uspenskii's nutrient medium [3]. The phages were introduced into the medium and kept in the same conditions as in all subsequent experiments. The initial concentration of phage corpuscles in the medium was determined accurately by control seedings on the day the experiments were carried out. Subsequent seedings were made every 3 days. The degree of inactivation of the phages was judged from the decrease of their biological activity, i.e., by the decrease in the number of active corpuscles (Table 1).

It is clear from Table 1 that on the first days of the experiment, considerable mortality occurred along all three phages, and on the 15th day the number of active corpuscles was only 4-9% of the original, after which their concentration remained almost unchanged. In subsequent experiments the changes in the biological activity of the phages were studied in analogous conditions, but in the presence of developing cultures of algae. The pH, measured on the day of the control seedings, was 6.9-7.7 (Tables 2-5).

\*The authors are grateful to N. I. Rybakov and V. V. Pantyukhova, on the staff of this Laboratory, for their generous assistance with the work.

TABLE 1. Preservation of Moderately Virulent ( $\lambda$ ) and Virulent ( $T_4$  and  $T_1$ ) Bacteriophages in Uspenskii's No. 1 Medium

Strain	Number of active corpuscles per ml						
	1 day	3 days	6 days	9 days	12 days	15 days	20 days
$\lambda$	16 000 (100)	10 500 (65)	7 200 (45)	3 000 (20)	1 100 (8)	600 (4)	500 (3,3)
$T_4$	230 000 (100)	110 000 (50)	52 000 (23)	36 000 (15)	28 000 (10)	22 000 (9)	20 000 (8)
$T_1$	200 000 (100)	90 000 (45)	60 000 (30)	51 000 (25)	37 000 (16)	14 000 (7)	13 000 (6,5)

Note. Here and in Tables 2-5 the percentages are given in parentheses.

TABLE 2. Inactivation of Bacteriophages by a Developing Culture of *C. vulgaris*

Test object	Number of algae and active phage corpuscles per ml					
	1 day	3 days	6 days	9 days	12 days	15 days
Algae Phage $\lambda$	500 000 16 000 (100)	2 000 000 9 000 (60)	3 250 000 2 500 (15)	4 300 000 600 (4)	6 200 000 0	7 000 000 0
Algae Phage $T_4$	500 000 230 000 (100)	2 000 000 110 000 (50)	3 250 000 52 000 (23)	4 300 000 36 000 (15)	6 200 000 28 000 (10)	7 000 000 22 000 (9)
Algae Phage $T_1$	500 000 200 000 (100)	2 000 000 80 000 (40)	3 250 000 2 000 (10)	4 300 000 4 000 (2)	6 200 000 90 (0,03)	7 000 000 0

TABLE 3. Inactivation of Bacteriophages by a Developing Culture of *S. obliquus*

Test object	Number of algae and active phage corpuscles per ml					
	1 day	3 days	6 days	9 days	12 days	15 days
Algae Phage $\lambda$	500 000 400 000 (100)	1 650 000 100 000 (25)	3 000 000 22 000 (5)	3 600 000 10 000 (2,5)	5 700 000 8 000 (2)	6 800 000 0
Algae Phage $T_4$	500 000 230 000 (100)	1 650 000 73 000 (32)	2 900 000 17 000 (7)	3 600 000 1 000 (0,4)	5 700 000 100 (0,01)	6 800 000 0
Algae Phage $T_1$	500 000 300 000 (100)	1 650 000 100 000 (30)	2 900 000 33 000 (10)	3 600 000 19 000 (6)	5 700 000 8 000 (3)	6 800 000 0

It is clear from Table 2 that the culture of the alga *C. vulgaris* caused considerable inactivation of the virulent phage  $T_1$ , and still more marked inactivation of the moderate phage  $\lambda$ . On the 12th-15th day no active corpuscles of these phages were found. Meanwhile, the alga had no effect on the virulent phage  $T_4$ .

Table 3 shows that the culture of the alga *S. obliquus* accelerated the inactivation of all three phages, and at the end of the experiment all phages had completely lost their activity. Sometimes as many as three such formations were found in one cell.

TABLE 4. Inactivation of Bacteriophages by a Developing Culture of *C. vulgaris*

Test object	Number of algae and active phage corpuscles per ml					
	1 day	3 days	6 days	9 days	12 days	15 days
Algae	1 000 000	2 200 000	3 600 000	4 250 000	5 100 000	5 900 000
Phage $\lambda$	1 400 (100)	750 (55)	240 (17)	120 (9)	80 (6)	28 (2)
Algae	1 000 000	2 200 000	3 100 000	4 400 000	5 100 000	5 200 000
Phage $T_4$	10 000 (100)	4 000 (40)	3 700 (37)	2 000 (20)	1 200 (12)	900 (9)
Algae	1 000 000	2 400 000	3 300 000	4 100 000	4 800 000	5 600 000
Phage $T_1$	10 000 (100)	6 000 (60)	4 200 (42)	2 500 (25)	800 (8)	300 (3)

TABLE 5. Inactivation of Bacteriophages by a Developing Culture of *S. obliquus*

Test object	Number of algae and active phage corpuscles per ml					
	1 day	3 days	6 days	9 days	12 days	15 days
Algae	1 000 000	2 300 000	3 200 000	3 900 000	4 400 000	5 100 000
Phage $\lambda$	1 400 (100)	740 (53)	300 (22)	180 (13)	90 (7)	50 (4)
Algae	1 000 000	2 400 000	2 950 000	4 600 000	5 600 000	5 800 000
Phage $T_4$	10 000 (100)	6 000 (60)	5 200 (52)	3 000 (30)	960 (10)	810 (8)
Algae	1 000 000	2 300 000	3 100 000	3 900 000	4 500 000	5 400 000
Phage $T_1$	10 000 (100)	7 000 (70)	5 000 (50)	4 100 (41)	3 000 (30)	1 500 (15)

To test the hypothesis that in natural conditions the interaction between phages and algae may be different, concentrations of phage corpuscles were taken close to those characteristic of reservoirs contaminated with sewage effluent (Tables 4 and 5).

The results in Table 4 show that only the developing culture of *C. vulgaris* caused slight inactivation of phages  $T_1$  and  $\lambda$ .

The results in Table 5 show that the developing culture of *S. obliquus* did not inhibit the phages although the initial concentration of algae in the last experiments was much greater than in the previous (Tables 2 and 3).

Hence, the fact observed by the authors previously [2] and in the experiments described above (Table 3), that the inactivating power of *scenedesmus* is greater than that of *chlorella* was observed only when the concentration of phage corpuscles was higher than usual. If, on the other hand, the concentration of phage corpuscles in the water was close to that naturally found, no inhibiting effect of the algae was observed. When the concentration of phages was increased, the algae caused marked inhibition of their biological activity, thus maintaining the equilibrium created in the reservoir between *E. coli* and its specific phages, so that the coli-titer was unchanged.

#### LITERATURE CITED

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