THE RELATIONSHIP BETWEEN ALGAE AND MICROORGANISMS

REPORT 3.* EFFECT OF THE ALGAE Chlorella vulgaris and Scenedesmus obliquus ON THE SURVIVAL OF BACTERIOPHAGES TO Salmonella typhimurium

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Enteric bacteriophage is known to take part in the processes of natural purification of rivers, as has been confirmed many times [2, 6]. It has frequently been observed that bacteriophages are a powerful factor restraining the spread of water-borne infections [3, 10]. Since they remain longer in water than microorganisms, bacteriophages can be used as a reliable indicator of the hygienic state of a reservoir [5, 8, 12]. For example, the discovery of Vi-phages in water is evidence of a source of typhoid infection [11].

Other factors taking part in the processes of self-purification of water supplies are phytoplankton organisms, which many workers regard as antagonists of bacteria. However, during the study of the relationships between bacteria and algae, it is not always possible to demonstrate antagonism. Sometimes when many bacteria are present in a reservoir, many algae can also be found [1].

The relationship between bacteria and algae is evidently complex. It is possible that phytoplankton not only acts directly on bacteria, but also affects the specific phages of bacteria. If algae cause the death of bacteriophages, they may by the same token create conditions in which the corresponding microorganisms may multiply.

EXPERIMENTAL METHOD

The investigation was carried out with four bacteriophages: K_1, K_2, T, and M. The phages were specific for Salmonella typhimurium and had multiplied on a reference strain (No. 2110) in meat-peptone broth. By their action on this strain, bacteriophages, K_1, K_2, and T caused lysis of the bacterial cells in a titer of 10^{-9}. Phage M lyzed these bacteria in lower titers (10^{-6}) and possessed lysogenic properties in relation to the reference strain. Considering their biological properties as a whole, three phages (K_1, K_2, and T) were virulent whereas phage M belonged to a moderately virulent type [4].

By the biological activity of the phage we meant the number of free corpuscles capable of giving negative colonies on a solid medium seeded with the reference strain. Seeding was carried out in meat-peptone agar by a two-layer method [9].

For the research we used algologically and bacteriologically pure cultures of the green anticoccal algae Chlorella vulgaris and Scenedesmus obliquus. The algae were maintained on Uspenskii's medium, as we have previously described [7]. The yield of algae was determined nephelometrically, and then converted to dry weight (in mg/100 ml of medium), and the pH was measured by means of a type LP-4 potentiometer with a glass electrode. The experiments were conducted at 18-20° and in an intensity of illumination of 2000-25,000 lx, provided by luminescent lamps of type BS-30 (e).

EXPERIMENTAL RESULTS

In preliminary experiments the degree and duration of the decrease in titer of the bacteriophages were studied in an experimental medium without algae. For this purpose, a precisely determined number of each of the phages was introduced into sterile Uspenskii's medium (pH 7.2), which was then submitted to the experimental conditions.

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The initial concentration of phage corpuscles in the medium was accurately determined by control seedings on the day the experiment was set up. Subsequent seedings were made every 4 days. The degree of inactivation of the phages was judged by the lowering of their biological activity, i.e., by the decrease in the number of active corpuscles. The results of these preliminary experiments are shown in Table 1.

The results in Table 1 show that during the first days of the experiment a considerable decrease took place in the numbers of all the phages, and on the 7th day the number of active corpuscles was not more than 17% of the original. After the initial sharp fall in the activity of the virulent phages K_1, K_2, and T, the concentration of corpuscles became more or less stable and control seedings in the later stages (until the 15th day) gave identical results. The activity of the moderately virulent M phage fell steadily throughout the period of observation to a value of less than 1% on the 15th day.

TABLE 1. Rate of Decay of Bacteriophage on Uspenskii's Medium without Algae

Phage strains	Periods of observation (in days)				
	1st	3rd	7th	11th	15th
	No. of active corpuscles in 1 ml				
K_1	12 500 (100%)	5 000 (40%)	2 000 (16%)	2 000 (16%)	2 000 (16%)
K_2	32 000 (100%)	8 000 (25%)	5 000 (15%)	4 800 (15%)	4 500 (14%)
T	35 000 (100%)	12 500 (35,7%)	6 000 (17%)	6 000 (17%)	5 000 (14%)
M	37 000 (100%)	24 000 (67,8%)	3 000 (8%)	1 500 (4%)	300 (0,8%)

TABLE 2. Inactivation of Bacteriophages in a Developing Culture of the Alga Chlorella vulgaris

Test object	Time of observation (in days)				
	1st	3rd	7th	11th	15th
	No. of active corpuscles in 1 ml and yield of algae (mg/100 ml)				
Phage K_1	12 500 (100%)	5 000 (40%)	3 000 (24%)	3 000 (24%)	3 000 (24%)
Algae	1,2	5,1	6	9	10,9
Phage K_2	32 000 (100%)	11 000 (34%)	8 000 (25%)	5 000 (15,6%)	4 000 (12%)
Algae	1,2	6	6,9	12,2	14
Phage T	35 000 (100%)	11 050 (31,4%)	6 000 (17%)	5 000 (14%)	4 000 (11,4%)
Algae	1,2	7,1	10,8	14,5	16,0
Phage M	37 000 (100%)	20 000 (54%)	0	0	0
Algae	2	3,5	5,2	12,4	14,5

In later experiments we studied the changes in the biological activity of the phages in the same conditions but in the presence of developing strains of algae. The pH values during proliferation of the algae in buffered Uspenskii's medium varied between 7.2 and 8.0. The results of two typical experiments are shown in Tables 2 and 3.

It will be seen from Table 2 that in the presence of a culture of Chlorella vulgaris the general pattern of inactivation of the virulent phages K_1, K_2, and T remained the same as in the medium without algae (Table 1). Irrespective of the increase in the concentration of the algae, these phages lost little of their activity during the second week of observation. The concentration of their corpuscles was 11-24% of the original value at this period. The moderately virulent M phage was inactivated to a greater degree in the presence of a culture of Chlorella vulgaris. On the 7th day of the experiment no active corpuscles of this phage could be seen in the samples.

It follows from Table 3 that the culture of the alga Scenedesmus obliquus accelerated the inactivation of all 4 phages. The number of corpuscles at the end of the observation was 0.3 and 0.005% respectively for phages K_2 and T, and none could be detected of phages K_1 and M. Hence, in the experimental conditions the virulent phages K_1, K_2, and T lost practically the whole of their activity in the presence of the alga Scenedesmus obliquus, but their activity was unchanged in the presence of the alga Chlorella vulgaris. The moderately virulent M phage was inactivated equally and completely under the influence of both algae.

TABLE 3. Inactivation of Bacteriophages in a Developing Culture of the Alga Scenedesmus obliquus

Test object	Time of observation (in days)				
	1st	3rd	7th	11th	15th
	Number of active corpuscles in 1 ml and yield of algae (in mg/100 ml)				
Phage K_1	12,500 (100%)	2,500 (20%)	0	0	0
Algae	1.8	5.8	11.5	22.4	25
Phage K_2	32,000 (100%)	12,000 (37%)	7,000 (22%)	1,000 (3%)	100 (0.3%)
Algae	1.8	4.2	12.2	20.9	26.2
Phage T	35,000 (100%)	10,000 (28.5%)	400 (1.1%)	100 (0.3%)	2 (0.005%)
Algae	1.8	5.1	12.4	17.8	21
Phage M	37,000 (100%)	1,850 (5%)	0	0	0
Algae	1.8	5	9.3	15	19.2

TABLE 4. Inactivation of Bacteria Contained in Cellophane Bags Immersed in Developing Cultures of the Algae Chlorella vulgaris and Scenedesmus obliquus

Test object	Time of observation (in days)				
	1st	3rd	7th	11th	15th
Bacteria (No. of cells in 1 ml)	40,000 (100%)	20,000 (50%)	8,000 (20%)	10,000 (25%)	6,300 (15.7%)
Chlorella (in mg/100 ml)	0.02	0.05	0.09	0.20	0.25
Bacteria	45,000 (100%)	24,000 (53.3%)	10,000 (22.2%)	1,300 (2.9%)	800 (1.8%)
Scenedesmus	0.02	0.05	0.09	0.25	0.3
Bacteria without algae	15,000	450,000	1,480,000	2,100,000	3,685,000

These biological objects may be observed to interact in a similar fashion in natural reservoirs. In this case it is obvious that pollution of the water with the microorganisms responsible for salmonelloses would be secured most rapidly by the presence of the alga Chlorella vulgaris: while depressing the multiplication of bacteria it does not inactivate the virulent phages and, consequently, does not interfere with the process of natural phagolysis.

In an attempt to elucidate the mechanism of action of the algae on the biological objects studied above, we carried out additional experiments. Suspensions of phages and bacteria were poured into cellophane bags and immersed in a medium with developing cultures of algae. The experimental conditions were as described above. The results of these investigations are given in Table 4. The depressing action of the algae was not prevented by the

layer of cellophane separating them from the bacteria and phages. It may therefore be suggested that the toxic secretions of the algae do not possess a high-polymeric structure, for they are capable of dialysis through cellophane.

Hence it follows that algae, by inactivating bacteriophages, may facilitate the development of the corresponding bacteria on a large scale.

SUMMARY

Virulent phages specific to Salmonella typhimurium lose their activity under the effect of Scenedesmus obliquus and show almost no change in the presence of Chlorella vulgaris. This makes it possible to explain the simultaneous presence of a considerable number of bacteria and algae in the water. Conditions may be created in the water reservoir where algae, depressing the development of the phages corresponding to bacteria, may become prevalent in the plankton.

The toxic products of algae metabolism were not of high polymeric structure.

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