VACCARO, R. F., BRIGGS, M. P., CAREY, C. L., AND KETCHUM, B. H. 1950 Viability of *Escherichia coli* in sea water. Am. J. Public Health, **40**, 1257–1266.

WAKSMAN, S. A. AND REILLY, H. C. 1945 Agar-streak method for assaying antibiotic substances. Ind. Eng. Chem., **17**, 556–558.

ZOBELL, C. E. 1936 Bactericidal action of sea water. Proc. Soc. Exptl. Biol. Med., **34**, 113–116.

An Evaluation of Factors Affecting the Survival of *Escherichia coli* in Sea Water[1]

IV. Bacteriophages

A. F. CARLUCCI[2] AND DAVID PRAMER

Department of Agricultural Microbiology, Rutgers, The State University, New Brunswick, New Jersey

Received for publication December 7, 1959

d'Hérelle (1926) suggested that bacteriophages contribute to the self-purification process in natural waters, but ZoBell (1946) reported that they occurred sporadically and only in the littoral zone and concluded that there was insufficient evidence for bacteriophages to be considered of importance in limiting the bacterial population of the open ocean. Nevertheless, it was repeatedly stated in the literature (Carlucci and Pramer, 1959) that bacteriophages contribute to the rapid death and paucity of bacteria in sea water. Recent studies (Kriss and Rukina, 1947; Spencer, 1955) have shown that bacteriophages are not limited to the littoral zone but occur at points distant from land and at depths as great as 2000 meters. The bacteriophage isolated (1955) and studied (1957) by Spencer was active against several strains of the luminous marine bacterium *Photobacterium phosphoreum*. It caused lysis of host cells on sea water agar but not on tap water agar and appeared to be indigenous to the sea.

The present report describes the occurrence, persistence, and activity of some bacteriophages in sea water.

MATERIALS AND METHODS

Various methods for the isolation of bacteriophages from sea water were tested, but the one that proved most successful was similar to that used in a study of the occurrence of bacteriophages in soil (Carlucci and Starkey, 1956). Portions of freshly collected samples of sea water were added to flasks that contained nutrient broth, and separate flasks were inoculated with cells of *Escherichia coli*, *Aerobacter aerogenes*, and *Serratia marinorubra*. The flasks were incubated for 48 hr on a rotary shaker[3] at 28 C after which their contents were filtered through Morton sintered glass discs of ultrafine grade (Morton, 1944). This primary treatment caused multiplication of phages present in the sea water sample, enriched the preparation, and increased the probability of phage isolation.

Each filtrate (1 ml) was added to a tube containing 5 ml of nutrient broth. The tubes were inoculated with appropriate host cells and incubated on a rotary shaker at 28 C. The presence of phage was expressed by clearing of the culture medium. When lysis was observed the contents of the tube were filtered through sintered glass and an aliquot of the filtrate was tested for its ability to produce plaques. To obtain plaque formation, approximately 10 ml of nutrient agar was added to a Petri dish and permitted to solidify. A 0.5-ml aliquot of the filtrate being tested was added to the surface of this agar layer and thoroughly mixed with an additional 10 ml of nutrient agar that were seeded with cells of the host bacterium and poured at approximately 45 C. When the second agar layer had solidified, the plates were incubated at 28 C for 24 hr and examined for plaques. Phage titers were measured by the same layering technique using aliquots of appropriate dilutions of filtrates. Plaque counts were made after 24 hr incubation and the number of phage units present in the filtrate was calculated from the plaque count, the volume of filtrate plated, and the dilution used. Procedures employed to measure the survival of

[1] Paper of the journal series, New Jersey Agricultural Experiment Station, Rutgers, The State University, Department of Agricultural Microbiology, New Brunswick, New Jersey. This investigation was supported in part by research grant E1437 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.

[2] Present address: Research Department, United Fruit Company, La Lima, Honduras, C. A.

[3] New Brunswick Scientific Instrument Company, New Brunswick, New Jersey.

E. coli in sea water were described previously (Carlucci and Pramer, 1960a).

Results and Discussion

Occurrence and persistence of bacteriophages in sea water. No difficulty was encountered in isolating phages active against *E. coli*, *A. aerogenes*, and *S. marinorubra* from samples of sea water collected approximately 600 feet offshore at Long Branch, New Jersey. Lysis of the marine bacterium by the phage took place in media prepared with sea water but no requirement for sea water was established. The phages active against *A. aerogenes* and *E. coli* produced plaques having a 2- to 3-mm diam whereas plaques formed by the phage active against *S. marinorubra* were approximately 1 mm in diam.

Persistence of the coliphage was studied by adding 5×10^7 phage units to each of a series of flasks containing 100 ml of the following waters: (a) 0.85 per cent NaCl in deionized water; (b) untreated sea water; (c) Millipore filter-sterilized sea water; and (d) autoclaved sea water. The flasks were stored at 28 C without agitation and phage titers were measured by making plaque counts at the time of inoculation and periodically thereafter for a period of 30 days.

The results listed in table 1 show that coliphages were most stable in autoclaved sea water and least stable in untreated sea water. Various investigators have shown sterilization to have a favorable effect on the survival of bacteria in sea water but no satisfactory explanation for the effect is available at this time (Carlucci and Pramer, 1959). More than 50 per cent of the coliphages added to autoclaved sea water survived for 30 days whereas the activity of coliphages in nutrient broth was reported to be decreased 20 per cent by storage overnight and to reach equilibrium at about 2 per cent of the original activity after 7 days (Ellis and Delbrück, 1939).

TABLE 1

Persistence of coliphage in various waters

Water	Survival		
	10 days	20 days	30 days
	%	%	%
Untreated sea water	2.0	<0.01	<0.01
Filter sterilized sea water	56.0	37.0	6.4
Autoclaved sea water	102.0	68.0	58.0
0.85% NaCl in deionized water	1.2	0.4	0.05

TABLE 2

Effectiveness of coliphage in sea water

Inoculum	Survival after 48 Hr	
	Escherichia coli	Coliphage
	%	%
Experiment 1		
E. coli, 1.2×10^6 cells/ml	6.3	
Coliphage, 5×10^5 units/ml		38.0
E. coli + coliphage	28.0	34.0
Experiment 2		
E. coli, 1.2×10^6 cells/ml	65.2	
Coliphage, 9.0×10^4 units/ml		55.6
E. coli + coliphage	27.6	—*

* Coliphage multiplied to 878 times the initial level.

Effectiveness of bacteriophages in sea water. Separate portions of sea water were inoculated with cells of *E. coli*, coliphages, and both coliphages and cells of the host bacterium. The phage inoculum was a filtrate of nutrient broth in which *E. coli* had developed and then undergone lysis. It contained soluble organic matter as well as phages. Since previous studies (Carlucci and Pramer, 1960b) showed that the survival of *E. coli* was influenced by organic nutrients, two experiments were performed. In the first, the phage inoculum was prepared using dilute nutrient broth (0.05 per cent peptone and 0.03 per cent beef extract), and in the second, conventional nutrient broth (0.5 per cent peptone and 0.3 per cent beef extract) was employed. In the former case, the concentration of organic nutrients in the water sample after addition of the phage inoculum did not exceed 8 ppm, whereas in the latter a maximum of 80 ppm was present. The lower concentration was not sufficient for extensive growth and multiplication of *E. coli* in sea water (Carlucci and Pramer, 1960b; ZoBell, 1946). The numbers of cells of *E. coli* and the phage titer present in the water samples after 48 hr incubation at 28 C were determined by colony and plaque counts, respectively. The results listed in table 2 show that in the absence of a significant amount of available organic matter (experiment 1) no increase in numbers of coliphages was observed and survival of cells of *E. coli* was not affected adversely. In the presence of sufficient organic matter to support growth and multiplication of *E. coli* in sea water (experiment 2) there was a marked increase in the phage titer and a decrease in cells of the host bacterium.

Although bacteriophages were isolated from and demonstrated to persist in sea water, their contribution to the death of bacteria that enter the ocean will depend on the nutrient status of the water. In areas of pollution, the organic matter content of sea water may reach levels that support bacterial growth. As a consequence phage multiplication will occur and may be of significance in reducing bacterial counts. Sea water that is free of pollution contains little dissolved organic matter and does not support extensive growth or rapid multiplication of bacteria. Under such conditions phages will have little or no effect on the survival of bacteria.

SUMMARY

Bacteriophages active against *Escherichia coli*, *Aerobacter aerogenes*, and *Serratia marinorubra* were isolated from sea water without difficulty when enrichment procedures were employed. Coliphages were inactivated rapidly in natural sea water but persisted in filter and heat sterilized sea water. The effectiveness of coliphages was dependent on the presence in sea water of sufficient nutrients to support growth and multiplication of host cells.

REFERENCES

CARLUCCI, A. F. AND STARKEY, R. L. 1956 Detection of phages in soil and other natural materials. Bacteriol. Proc., **1956,** 35.

CARLUCCI, A. F. AND PRAMER, D. 1959 Factors affecting the survival of bacteria in sea water. Appl. Microbiol., **7,** 388–392.

CARLUCCI, A. F. AND PRAMER, D. 1960a An evaluation of factors affecting the survival of *Escherichia coli* in sea water. I. Experimental procedures. Appl. Microbiol., **8,** 243–247.

CARLUCCI, A. F. AND PRAMER, D. 1960b An evaluation of factors affecting the survival of *Escherichia coli* in sea water. II. Salinity, pH, and nutrients. Appl. Microbiol., **8,** 247–250.

ELLIS, E. L. AND DELBRÜCK, M. 1939 The growth of bacteriophage. J. Gen. Physiol., **22,** 365–384.

D'HÉRELLE, F. 1926 *The bacteriophage and its behavior.* The Williams & Wilkins Co., Baltimore, Maryland.

KRISS, A. E. AND RUKINA, E. A. 1947 Bacteriophage in the sea. Doklady Akad. Nauk S. S. S. R., **57,** 833–836.

MORTON, H. E. 1944 A new style assembly for fritted filters. J. Bacteriol., **47,** 379–380.

SPENCER, R. 1955 A marine bacteriophage. Nature, **175,** 690–691.

SPENCER, R. 1957 Thermal inactivation of a marine bacteriophage. J. Gen. Microbiol., **16,** vi–vii.

ZOBELL, C. E. 1946 *Marine microbiology.* Chronica Botanica Co., Waltham, Massachusetts.

Etiology of Sour Pit of Peaches

M. A. SMITH

Market Quality Research Division, Agricultural Marketing Service, U. S. Department of Agriculture, Chicago, Illinois

Received for publication December 10, 1959

In 1956 peaches held for ripening at the U. S. Fruit and Vegetable Products Laboratory at Prosser, Washington, showed abnormal softening in stem-end cavities at the time of canning. When the fruit was cut open the flesh in the pit and adjacent areas appeared light-brown to brown and was watersoaked and slimy. A yeasty or sour odor generally accompanied these symptoms. Affected flesh when planted on nutrient glucose agar yielded an organism having the characteristics of a yeast (figure 1*A*). Pure cultures were obtained readily by serial dilutions on nutrient and yeast glucose agars.

Reports from a number of canning plants in the Yakima district indicated that ripening fruit showing unusual stem-end softening, and other symptoms, as described above, had been observed in previous years. The term "sour pit" had been used by processors as descriptive of the disorder. The same terminology will be used in this paper.

It is well known that fungi, yeasts, and bacteria are common contaminants of cannery wastes and of decaying fruits and vegetables. Since fruit flies are attracted by odors of fermentation and feed on such wastage, the possibility was suggested that in the large *Drosophila* population present, some might be acting as vectors for the sour pit organism. To determine this, flies were trapped in the laboratory near lugs of stored fruit, anesthetized, crushed, and planted on plates of nutrient glucose agar. Tissue plantings of fruit affected with the disease were also made to this medium. Within 48 hr, colonies of yeast had appeared at the sites of many of the tissue and *Drosophila* plantings. Bacterial and fungal contaminants occasionally accompanied the yeasts but following the dilution plate method, the organism was readily recovered in pure culture.

The following report contains the results of further studies of the pathogenicity, physiological characteristics, morphology, and taxonomy of the causal organism.

EXPERIMENTAL METHODS AND RESULTS

Pathogenicity. Several methods were used to test the pathogenicity of the sour pit organism. In the first method, 24 peaches in the firm-ripe stage of maturity were cut along their sutures to pit depth. Six fruits were inoculated by introducing a suspension in sterilized water of a 10-day-old malt extract culture, originally isolated from infected fruit, between the cut edges of the peaches. Six fruits were similarly inocu-