

tryptophan-independent recombinants were selected in the presence of streptomycin. The 19 recombinants tested from each of the three crosses were resistant to T1. When strain K-12 was used as the male, selecting in the same way, the 19 recombinants tested were T1,try<sup>+</sup>. This

indicates close linkage between the T1 locus and the T1,try locus in strain K-12, giving further support to the idea that the T1,try<sup>-</sup> mutation in K-12 is a simultaneous deletion of the T1 locus, and of some or all of the loci controlling tryptophan synthesis.

## INDIGENOUS MARINE BACTERIOPHAGES<sup>1</sup>

R. SPENCER

*Humber Laboratory, Department of Scientific and Industrial Research, Hull, England*

Received for publication November 24, 1959

The presence in sea water beyond the littoral zone of bacteriophages active against marine bacteria has not been conclusively demonstrated despite their possible importance in marine microbiology. Although ZoBell (*Marine Microbiology*, 1946) isolated phages from sea water of the littoral zone, he was unsuccessful with water taken beyond. The phages isolated by Kriss and Rukina (Rept. U. S. S. R. Acad. Sci., 57, 833, 1947) from the Black Sea were active against such typically terrestrial species as *Bacillus subtilis* and *Micrococcus albus* (Kriss *et al.*, Trans. biol. Sta. Sebastopol, 7, 50, 1949, and Kriss, *personal communication*) and may thus have been adventitious, whereas the phage isolated by Smith and Krueger (J. Gen. Physiol., 38, 161, 1954) against a marine vibrio was not strictly marine in origin. Consequently, attempts were made to isolate phages from sea water taken well beyond the littoral zone active against typically marine bacteria.

The sea water samples were taken from the North Sea, some 10 miles off Aberdeen, Scotland, and the marine bacteria consisted of two groups, strains of *Photobacterium phosphoreum* isolated from marine fish, and strains of several species isolated from a further sample of sea water. After preliminary experiments, two methods of isolation were used in parallel, one direct and one indirect, each with incubation at both 20 and 0 C.

The direct method consisted of layering on a nutrient agar base a mixture of 10 ml of 1.5 per cent nutrient agar, 10 ml of sea water previously membrane filtered to remove interfering bacteria,

and 2 ml of a dense suspension of the appropriate bacterial culture. After incubation, the presence of any phage active against that particular culture was detectable by plaque formation. Samples (600 ml) of sea water at both 20 and 0 C were normally examined at a time against a total of 40 strains of bacteria.

In the indirect method, 300 ml of sea water were mixed with 100 ml of quadruple strength nutrient broth and 8-ml amounts of broth culture of four different bacterial strains. After incubation, the bacteria were removed by either filtration or by an adaption of the chloroform technique of Fredericq (Compt. rend. soc. biol., 144, 295, 1950) involving carbon tetrachloride, and the bacteria-free preparation tested for lytic action against the appropriate bacterial strains by the above layer technique. Samples (3 L) of sea water at both 20 and 0 C were normally examined at a time, again with 40 strains of bacteria.

In all, 6 L of sea water were examined by the direct method and almost 40 L by the indirect method, and seven phages were isolated. Four of the phages were detected by the parallel direct and indirect methods at both 20 and 0 C; three were present in samples in a concentration of 1 to 5 particles per 10 ml, and one in a concentration of approximately 100 particles per 10 ml. The remaining three phages were detected by the indirect method.

One phage was active against a strain of *Photobacterium phosphoreum*, three against unidentified nonpigmented *Pseudomonas* species, two against an unidentified Flavobacterium, and one against an organism provisionally classified as a *Cytophaga* species.

<sup>1</sup> The work described in this paper was carried out as part of the program of the Department of Scientific and Industrial Research.