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Preliminary Report

Isolation of UV-inducible Temperate Phage ϕ80

Recently Jacob (1955, 1956)[1,2] isolated about 30 different coliphages lysogenizing on *Escherichia. coli* K12. In the lysogenic cells of *E. coli* K12, various prophages are located on specific sites of the host chromosome. The UV-inducible and non-inducible prophages are located on quite different regions of it.

The validity of these facts in other prophages was therefore of interest and was the subject of this study. After isolating a UV-inducible phage, lysogenizing on *E. coli* K12, some of its properties were studied.

In this series of isolation experiments, the author has isolated on SS-agar plates 564 pure cultures of *E. coli* from almost 3,000 test samples of *Shigella-Salmonella* brought to the Department of Clinical Examination of our Institute between April 1 and April 15, 1960. Agar plates were coated with a W3110 (K12 wild type, non-lysogenic for λ) culture and dried. The isolated liquid cultures described above were spotted at a rate of 25 per plate. After the plates had been induced by weak UV-light treatment, they were incubated overnight. Lysogenic or colicinogenic cultures formed transparent inhibition zones on a white background. Nine cultures forming clear zones were selected for further study and the production of phages and colicine was investigated. Only one of nine, #80 isolates B80 (ϕ 80)+, produced a turbid inhibition zone even after treatment of the samples with trypsin or nagase (a protease from *B. subtilis*). The remaining eight lysates were therefore from colicinogenic strains.

B80(ϕ80)+ is a typical *E. coli*, being prototrophic, streptomycin sensitive and not fermenting maltose. The results of inspection tests are summarized as follows:

Kliegler A/AG; SM−/AG; H_2S, Ind., Mov., −++;

Cit. −; VP − ; MR + ; U − ; etc.

This strain shows no reaction with λ phage. Moreover, from the fact that this strain does not yield recombinants with K12 F+ bacteria on appropriate selection media, this strain can apparently not be crossed with strain K12.

To obtain a non-lysogenic strain of B80, severe UV-irradiation treatment of lysogenic B80(ϕ80)+ was carried out. One tenth ml of the lysogenic culture (containing about 5×10^4 cells) per plate was irradiated (19 watt, 80 cm, 45″). Lysis occurred and only about 10−100 colonies per plate survived. Some of the colonies which survived has a cataract-like appearance. They were purified by repeated single colony isolation. Most of them were ϕ80 free non-lysogenic strain B80 (ly)−, having lost their immunity to ϕ80.

ϕ80 gives a turbid plaque with a central ring of secondary growth and a halo on K12 strains but only a small one on B80 (ly)−. The plaque size of ϕ80 on K12 is more uniform than that of wild type λ^{++}, and is about the same as that of λv_2.

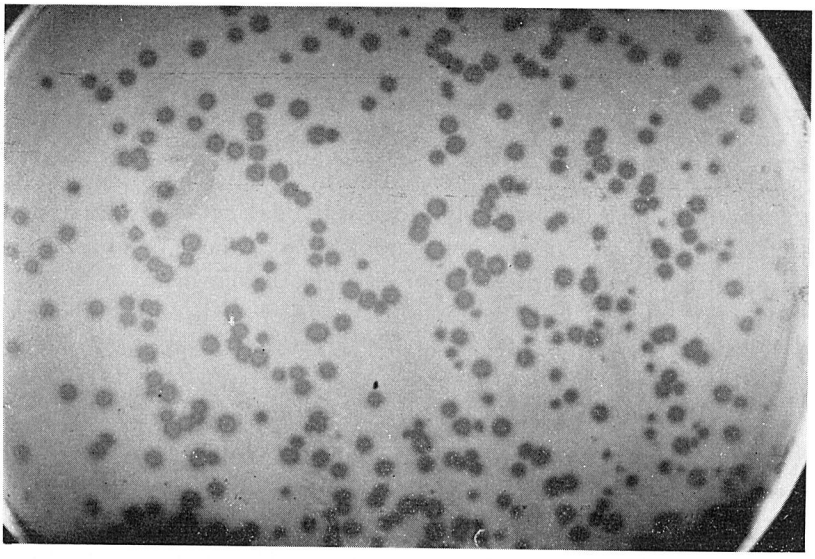

Fig. 1. Plaque Morphology of Phage ϕ80 on Strain K12

The phage titer of ϕ80 lysates derived from K12, described as ϕ80 [K12], was much higher on K12 than B80 and conversely ϕ80 [B80] had higher plaque efficiency on B80 than K12 (see Fig. 2). The plaque efficiency of the phage depends upon its source and is independent of the nature of the lytic or inducible preparation (Table 1). These relationships may be the result of host controlled variation.

The serological and genetical interrelation between ϕ80 and λ has not yet been studied in detail. But by this time we can not find any fact for this problem. However, many other techniques (*e. g.* chloroform treatment for pasturization and fractionation with $(NH_4)_2SO_4$) used for λ were also possible with phage ϕ80.

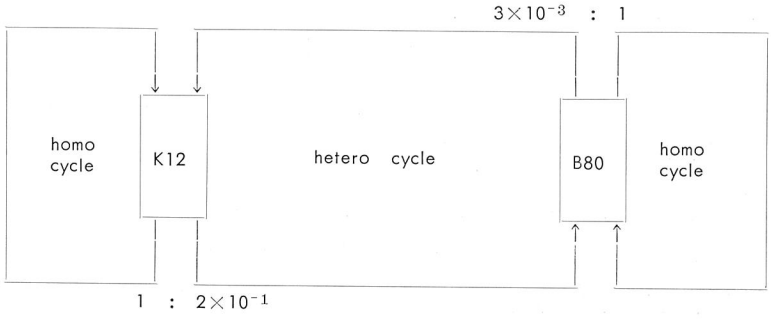

Fig. 2. Host Controlled Variation in ϕ80

Lysates containing a high titer of vegetative phage particles were prepared by a similar method to that used for phage λ from lysogenic K12 (ϕ80)+ after UV-irradiation treatment (Weigle and Delbrück, 1951).[3] Aerated broth cultures of

Table 1. Comparison on e. o. p. of Phage ϕ80 Derived from Various Sources by Various Techniques

source	phage lysate	Plaque counts on		e. o. p.*
		B80	K12	
ϕ80[B80]	inducible	1.7×10^{6}	8×10^{5}	2×10^{-1}
ϕ80[B80]	lytic	6×10^{9}	8×10^{8}	1.3×10^{-1}
ϕ80[K12]	inducible	1.5×10^{8}	4.5×10^{10}	3×10^{-3}
ϕ80[K12]	lytic	1.5×10^{8}	5.0×10^{10}	3×10^{-3}

* titer on directly derived cell = 1

the lysogenic strain (ca. 10^9 cells per ml) were centrifuged, and the cells were resuspended (ca. 5×10^8 cells per ml) in 0.5 per cent saline. The cell suspension (4 ml) in a petri dish (diameter 9 cm) was exposed to UV-light (19 watt, 40 sec., at 80 cm). After irradiation the suspension was diluted with the same volume of double-strength broth medium and aerated at 37°C until maximal clearance of the broth was obtained. This usually required 3 hours. Induced lysates usually have plaque titer of approximately 5×10^{10} particles per ml.

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