## STUDIES OF A BACTERIAL VIRUS ACTIVE AGAINST XANTHOMONAS PRUNI (HOLMES)[1]

RUTH CROWNOVER, J. D. MANDEL and A. EISENSTARK,
Oklahoma A&M College, Stillwater

*Phagus pruni* (Holmes) is a virus which is widespread in the United States, being associated with its host organism *Xanthomonas pruni*, a bacterium of considerable economic importance as the cause of bacterial rot of peaches. In spite of its common occurrence, however, the phage has not been studied to any great extent (3).

Standard nutrient agar, semi-solid agar, and broth were used in all the experiments, except the work with synthetic media.

The methods used varied according to the experiment, but in all cases the layering method of plaque counts described by Delbruck (2) was used for quantitative determination of virus particles.

One of the first phases of the experimental work was concerned with the effect of some of the physical and chemical agents on the growth of the virus.

A study of the effect of temperature revealed that *Phagus pruni* (Holmes) was slightly inactivated at 65° C., but the actual temperature of inactivation was 70° C. for 10 minutes. *Phagus pruni* retained 10% of its activity in the presence of 6 molar (30%) ethyl alcohol and was completely inhibited by 20 molar (90%) ethyl alcohol. Phenol completely inactivated the virus in concentrations greater than 0.5 molar and had no effect on it in concentrations less than 0.3 molar (Fig. 3). *Pruni* phage is slightly inhibited by 10 and 15 minutes exposure to an ultraviolet lamp, almost completely inactivated after 30 minutes, and its lytic effect completely destroyed by one hour's exposure. In studying the effect of *p*H it was revealed that the bacteriophage was active at all the hydrogen-ion activity values that allowed the growth of the host bacterium. Growth of the organism was not observed at a *p*H lower than 5.2 or higher than 8.0.

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An experiment was run to supplement the work of Thornberry, Braun and Elrod (5) who found the phage to be active against *X. pruni* but non-active against other related plant pathogens. The supplemental tests included exposure of *Salmonella, Shigella, Escherichia, Pseudomonas, Serratia,* spore-formers, and cocci to the virus. No lysis was observed. Because of its apparent specificity, it is possible that the phage may be used to identify an unknown organism as to whether or not it is *X. pruni.*

Another experiment concerning the characteristics of the phage was the determination of burst time, i.e., the time required for the phage to cause the bacterial cells to burst. Trials indicate a burst time of 2 hours as contrasted with the *T* system of phages attacking *E. coli* which has a burst time between 12 and 20 minutes (2).

The experimental work in progress at the present time is concerned with three phases: (1) the separation of types of *Phagus pruni* (Holmes) according to the type of lytic area or plaque produced on a film of the susceptible organism grown on a solid medium, like the separation of types of *E. coli* phages (2); (2) the accessory growth substances affecting the reproduction of pruni phage; and (3) the study of a possible lysin in semi-cleared areas observed around some plaques which is similar to the C lysin reported by Humphries (4) in semi-cleared areas surrounding plaques produced by bacteriophage active against *Klebsiella pneumoniae* Type A.

The experiments on the separation of types of *pruni* phage by observing the plaques produced from suspensions of single plaques have so far revealed 5 plaque types which maintain their distinctive characteristics from generation to generation. An interesting sidelight of these experiments was the observation of small clear-appearing areas of various sizes which looked superficially like plaques. Microscopic observation of these areas and unfruitful attempts at transmission showed them to be the result of bubbles on the agar surface. When counting plaques, one might easily mistake these clear areas for plaques and so obtain erroneous counts.

The work on accessory growth substances is as yet too immature to report results. Possible tryptophane requirement is first being tried (1).

The attempted study of a lysin in the semi-cleared areas or halos surrounding plaques has shown that spot inoculations of a sterile filtrate of one halo type plaque producing phage, which will lyse an active culture of *X. pruni* in dilution of 1:100, will produce observable clearing on films of the adult host organism. Further experiments with other phage types which also produce a halo type of plaque are being carried out as well as the attempted separation of this lysin from the bacteriophage.

## Summary

Bacterial viruses or bacteriophages provide a convenient means of studying viruses in general. Experimental investigation on *Phagus pruni* (Holmes) pathogenic to *Xanthomonas pruni* included studies of the inactivation of the phage by several agents and the burst time of the phage. The experimental work in progress is concerned with: (1) the separation of types of *Phagus pruni* (Holmes), (2) accessory growth substances affecting the reproduction of pruni phage, and (3) the study of a possible lysin from halo-type plaques.

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