A BACTERIOPHAGE FOR PSEUDOMONAS PYOCYANEA
(PSEUDOMONAS AERUGINOSA)

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In a preliminary report the isolation of a bacteriophagelike principle for *Pseudomonas pyocyanea* (*Pseudomonas aeruginosa*) was recorded (Fastier, 1945). Although numerous studies have appeared on phage action upon various organisms, none have mentioned the occurrence of such a principle for *P. pyocyanea*. In the present work a more complete description of phage activity for this organism is given.

Bacteriological examination of feces from a patient with acute colitis showed the normal coliform flora and several abnormal colonies of *P. pyocyanea*. These colonies exhibited the usual slight metallic sheen after overnight incubation on the MacConkey medium used for routine plating of feces. Adjacent colonies became confluent. There was no tendency toward abnormal spreading or departure from the normal butyrous consistency. Slight discoloration by pyocyanine was apparent even after such a short incubation period.

Subculture of the affected colonies on nutrient agar showed a plaque formation similar to that produced by phage Q151 on strains of *Eberthella typhosa* (Craigie and Brandon, 1936). After 18 hours of incubation these plaques were most numerous in confluent areas of growth; the majority measured 2 mm, and very few were more than 3 mm, in diameter. After 24 hours of incubation secondary growth became visible and later took the form of small papillae in the centers of the original plaques.

Preparation of a crude phage filtrate involved the Seitz filtration of a 6-hour trypsin broth culture of the original organism containing the phage plaques. No viable bacteria could be demonstrated in the filtrate by subsequent culture.

A strain of *P. pyocyanea* which had been maintained as a stock culture for several years and which showed variable pyocyanine production was used in the preliminary phage titration. No plaque formation was in evidence; the strain showed the typical morphological and biochemical reactions of *P. pyocyanea*. This organism is designated *Ps.2*. In the titration, a young broth culture of *Ps.2* was added to serial dilutions of the active filtrate. Removal of one 3-mm loopful from each dilution tube to an agar medium was carried out at 2-hour intervals over an incubation period of 12 hours at 37 C.

The results of this titration indicate that the phage shows activity in a titer of $1 \times 10^{-8}$ after 8 hours of growth. Areas of lysis were not complete, there being colonies of resistant organisms within the plaques. Secondary growth occurred after 20 hours. Several phage filtrates were prepared from single colonies of *Ps.2* after treatment with the crude lytic filtrate. The results heightened the
contention that two strains of varying resistance to phage action were present in the culture. Titration figures for six such filtrates against the original Ps.2 organisms showed activities of $1 \times 10^{-3}; 1 \times 10^{-4}; 1 \times 10^{-4}; 1 \times 10^{-3}$; and $1 \times 10^{-10}$. In all cases the areas of lysis were not sharply demarcated owing to the presence of varying numbers of resistant colonies.

Isolation of these resistant and nonresistant forms from the organism Ps.2 involved the preliminary phage selection followed by continuous subculture and phage treatment of single colonies. The final products after several hundred selections were a strain of Ps.2 totally resistant, and another completely susceptible to the bacteriophage, designated Ps.2A and Ps.2B, respectively.

Owing to the variable nature of pyocyanine production in the parent organism, Ps.2, observations on the formation of this pigment by strains Ps.2A and Ps.2B were not considered justifiable.

The two strains were examined for pyocyanase production by employing both the cup plate and the Oxford cylinder methods. Broth cultures of the organisms were used, and their antibiotic activity towards Staphylococcus aureus (Oxford H) was determined after 5, 10, 18, and 24 hours at 37 C. Owing to considerable outgrowth of the organisms from the cups a series of Seitz-filtered cultures of Ps.2A and Ps.2B were employed. It was later found that the antibiotic activity was not destroyed at the thermal death point of the organisms. Cultures were heated to 85 C for 15 minutes before measurement into the cups of the assay plate. The zones of inhibition resulting from the use of this procedure were clear cut and thus easily measurable. It was found that slight stimulation of the staphylococcal colonies occurred in the neighborhood of cups containing unheated 5-hour broth cultures of either strain. This stimulating effect on growth was abolished by heat. Apart from small discrepancies which could be ascribed to experimental error, no differences in pyocyanase production were detectable in either of the two strains.

A filtrate prepared from a young culture of Ps.2B and showing activity in $1 \times 10^{-10}$ was employed in an endeavor to ascertain whether or not the phage was specific for P. pyocyanea. Organisms recently isolated, and others maintained as stock cultures, were grown in the form of young broth cultures and then spread over the surface of agar plates. After the fluid phase had been absorbed, a 3-mm loopful of the bacteriophage was inoculated in the center of the bacterial spread. The results were recorded after 18 hours of incubation at 37 C. No lytic effects were observable in cultures of the following organisms: Salmonella paratyphi A (4 strains), Salmonella paratyphi B (4 strains), Salmonella paratyphi C (1 strain), Salmonella newport (1 strain), Salmonella gallinarum (1 strain), Salmonella pullorum (1 strain), Salmonella enteritidis (6 strains), Salmonella aberdeen (1 strain), Salmonella cholerae-suis (2 strains), Salmonella aertrycke (2 strains), Eberthella typhosa ("V" and "W" forms), Shigella dysenteriae (Shiga), Shigella paradysenteriae (Sonne), Shigella paradysenteriae (Schmitz), Shigella paradysenteriae (Flexner), Alcaligenes faecalis, Chromobacterium prodigiosum, Aerobacter aerogenes (2 strains), Escherichia coli (10 strains), and the paracolon group (4 strains).
SUMMARY

The isolation of a bacteriophage specific for *Pseudomonas pyocyanea* (*Pseudomonas aeruginosa*) is reported. By means of this phage a stock culture of *P. pyocyanea* has been shown to be composed of resistant and nonresistant strains, which up to the time of writing have shown no variation in their reaction to phage treatment. No difference in pyocyanase production by the two strains could be detected.

REFERENCES
