A PRELIMINARY STUDY OF THE BACTERIOPHAGES OF *PSEUDOMONAS AERUGINOSA*

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CONTENTS

I. INTRODUCTION

During the routine study of strains of *Pseudomonas aeruginosa* from various pathological sources, we have frequently noticed, especially in strains producing green pigment, depressed plaque-like areas covered with a metallic sheen. These are seldom seen in young cultures, although pigment may be clearly evident, and do not occur on MacConkey or S.S. medium.

The phenomenon has been repeatedly described. Hadley (1924) attributed it to a 'transmissible lytic agent'. He noted its disappearance when cultures lost their ability to produce pyocyanin. Strains which lost the metallic effect also lost other characteristics, such as rapid growth and proteolytic power. When present, the metallic effect persisted on subculture, but supernatant fluids from broth cultures did not increase the metallic effect, suggesting that no transmissible agent was free in them.

Filtrates from 'metallic' cultures added to 'non-metallic' cultures produced apparently typical bacteriophage plaques without a metallic sheen. From our own work, we consider that such plaques may not be related to the metallic effect.

Asheshov (1926) also isolated true bacteriophages without establishing their relationship to the metallic appearance. Linz (1938) showed that the metallic lytic effect is not related to antigenic constitution. Conge (1948) concludes

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that the metallic areas and bacteriophage plaques are different phenomena. She did not succeed in inducing metallic plaques by the addition of filtrates from metallic strains to cultures of non-metallic ones. Such filtrates as well as those from non-metallic strains did, however, contain true phages.

D'Herelle (1926) concluded from his own work and that of others that the metallic areas are caused by a principle which lacks the characteristics of the bacteriophage but which is associated with the function of pigment production.

Among our strains, one produced red pigment. Such red strains appear to be relatively rare. They have been studied by Gessard (1919) and Meader (1925).

Our strains were very variable, particularly in pigment production. Yellow fluorescent strains are usually considered to be variants of the green pyocyanin-producing strains. Gaby (1946) described three types: (i) a yellow fluorescent, (ii) a green type which may show the metallic lytic effect and (iii) a variable type, usually rough. He did not include a red strain in his series.

Bacteriophages against *Pseudomonas* have been isolated by Hadley (1924), Jadin (1932), Rabinowitz (1934), Fastier (1945) and Dickinson (1948), from various sources, e.g. filtrates of faeces, and of pus, but chiefly from lysogenic strains of *Pseudomonas*.

Our first intention was to investigate the metallic lytic effect so frequently encountered in the *Ps. aeruginosa* strains isolated during routine diagnostic work. In addition we are making a systematic study of the bacteriophages present in these cultures.

II. SOURCES AND BACTERIOLOGICAL PROPERTIES OF STRAINS

The sources of thirty strains available for study, as well as their appearance on first isolation, are listed in Table 1. Preliminary tests showed the presence in

Table 1

		Sources				
Appearance of strain	Nos.	Urines	Faeces	Pus	Rats*	Stock
Green, metallic	16	7	3	6†	—	—
Green, non-metallic	4	1	—	1	2	—
Yellow, non-metallic	10	7‡	1	—	—	2
Totals	30	15	4	7	2	2

* Strains isolated from cervical abscesses of laboratory white rats.

† One of these strains on first isolation produced a green pigment, but on prolonged incubation developed a deep purple colour.

‡ One of these yellow strains on prolonged incubation developed a deep red colour.

cultures of the green pigment producers of pyocyanin and fluorescin, whilst the yellow pigment was probably fluorescin only. The red pigment produced by the one strain appeared to consist, at least in part, of a substance differing from pyocyanin in solubility and reaction to change of pH. It differed also from the brown discoloration which occurred on prolonged storage of the usual pyocyanin producing strains.

In 24-hr. cultures all were small, motile, Gram-negative bacilli.

Stocks were maintained by monthly subculturing on Hartley's agar slopes, incubating at 37° C. for 2–3 days and then storing at room temperature. These remained viable for 6 months or more.

(*a*) *Cultural characters on solid media*

Colonies on 1 or 2% Hartley's agar were mainly of two types. The commoner type was slightly raised, with irregular fimbriate edges, about 1–2 mm. in diameter after 48 hr. incubation. The shape varied with the age. The surface was usually dull and granular. This type of colony was usually given by the pyocyanin-producing strains, and was the type commonly associated with the metallic effect.

The second type of colony was much smaller, about $\frac{1}{2}$–1 mm. in diameter, more compact, rounder, with an entire edge and a shiny surface. It was given by the yellow fluorescent or colourless strains only, and was usually devoid of metallic effect. Both types were easily emulsifiable.

Pigment production occurred under aerobic conditions, was fairly good under 10% CO_2, but absent in anaerobic cultures. Pigment was produced more rapidly at 37° C. than at room temperature. After 18 hr. a pale green or yellow colour began to diffuse into the surrounding medium, pigmentation increasing with further incubation. In the pyocyanin-producing strains this produced a deep, almost blue-green, or, in one strain, a purple colour. The yellow pigment also deepened to a fluorescent golden yellow, especially in one strain in which a few days later it became a clear, deep red.

The green and purple strains on prolonged incubation changed to a deep, rather murky brown, the yellow to a lighter brown, but the red retained the same clear colour over long periods.

Young cultures possessed a characteristic 'mousy' smell which in older cultures became ammoniacal.

The metallic effect was best shown on solid media, e.g. Hartley's agar plates, heavily sown by flooding the surface with young broth cultures. It was sometimes obvious in 18 hr. cultures, but was at its best after 48 hr. incubation. The first sign was a slight depression on the surface of the culture, often with a small central papilla, about 1 mm. in diameter. The metallic lustre usually started about the centre of the papilla, spreading over the rest of the 'plaque', but not to the surrounding, apparently normal, growth. It was typically iridescent, with a silvery gold or multi-coloured sheen. In one strain the metallic plaque was larger ($1\frac{1}{2}$ mm.), and the metallic film tended to spread out radially. Yet another strain differed in that the 'plaque' itself showed no iridescence, but was surrounded by an iridescent 'halo' about $\frac{1}{8}$ in. wide. Where the 'plaques' ran together, the 'halo' showed only round their edges.

If the 'plaques' occurred near the edge of an area of confluent growth, the wall nearest the edge broke down, and the lytic and metallic effect spread out in a wedge or fanlike manner. In some cultures areas of confluent bacterial growth might show only a few central metallic 'plaques', whilst at the periphery the metallic appearance was present in the form of an almost continuous ring about $\frac{1}{8}$ in. wide.

In older cultures with marked metallic effect the growth of the *Pseudomonas* became flattened out under the metallic coating, though the growth beyond was not affected. Where the 'plaques' ran together to form large flattened iridescent areas, irregularly shaped 'islands' of apparently normal growth were left here and there.

The metallic effect was noted in the green-, purple- and red-pigmented strains, but only one yellow-pigmented strain, a variant of an originally green one, showed it. Where the metallic effect was marked, pigment production was slow, and only after some days spread through the medium under the affected area from less metallic areas of the growth. The extent of the metallic sheen increased gradually for several days, both at room temperature and at 37° C.

Both pigment production and the development of metallic areas were well marked also on blood agar, boiled blood agar, glucose agar, lead acetate medium, and phenol-red urea medium. On Sabouraud's maltose medium and on Dorset's egg medium, neither pigment nor metallic effect was marked, though growth was good. On MacConkey's and S.S. medium the colonies had the appearance of non-lactose fermenters, and produced pigment changing the colour of the medium to a brown, but did not produce the metallic effect. Subculture from both types of media always resulted in the development of metallic colonies from originally 'metallic' strains. On S.S. medium the colonies were rounded and compact and of a rubbery consistency. After 48 hr. the growth became blackened and developed a generalized sheen, even with the non-metallic strains. This was non-lytic in character and quite different from the true metallic effect.

On 2% glycerine agar growth was heavy, raised and wrinkled.

(*b*) *Cultural characters on fluid media*

In broth all strains produced marked turbidity and a surface pellicle in 18 hr. Pigment production was always slower than in solid media, and started at the surface. If the cultures were shaken they rapidly became coloured throughout, because of the oxidation of the colourless leuco-base of the pigment in the depth of the medium. Strains producing pyocyanin and fluorescin showed their respective colours in fluid cultures. The strain producing a purple colour on solid media showed a very deep green colour in fluid media. The red strain also showed a very deep fluorescent yellow in most fluid media, becoming faintly red only on prolonged incubation in serum broth or glucose broth.

With all metallic strains, the surface pellicle showed a slight iridescent sheen, and on occasions even 'plaques'.

Broth cultures of some strains became very viscous, but the viscosity could not be correlated with either pigment production or the metallic effect.

(*c*) *Biochemical properties*

All strains produced acid in glucose, some in 3 days, other strains taking longer. Lactose, sucrose, mannite and maltose were not fermented, but pigment production in these sugars was good, except in the case of the red strain. The metallic

effect could be seen in the pellicles. Gelatin was liquefied, more rapidly by the deeply pigmented strains; nitrates were reduced to nitrites in 24 hr.; the methyl-red test was negative, and hydrogen sulphide was produced in lead acetate medium. Milk was peptonized by all strains, but more slowly by the yellow or colourless variants. The test for indole was positive in pyocyanogenic strains using the usual Ehrlich's technique, but tests on ether extracts of the cultures were negative. All strains grew well in Koser's citrate medium, but with little pigment production.

(*d*) *Pigment production*

A variety of liquid and solid media were tested for speed and amount of pigment production. The liquid media included Hartley's broth, Lab-Lemco broth, synthetic media, tryptone water, glucose broth, serum broth and 2% glycerine broth; for solid media 1 and 2% agar bases were used.

The Lab-Lemco base and tryptone were found less favourable for pigment production than veal infusion.

The synthetic medium used by Delbrück (1939) in *B. coli* phage studies was found satisfactory, though growth in this medium was slow.

Pyocyanin production was good when glycerine was added to the broth or agar, but poorer when glucose or serum was added. Fluorescin production, on the other hand, appeared to be better in the presence of glucose or serum and less with glycerine. The purple strain produced pyocyanin only in fluid media, and on solid media the purple colour was produced best in the presence of glycerine. The red pigment was also poorly developed in fluid media, only serum or glucose in Hartley's infusion broth showing a slight red colour after 14 days. The addition of glycerine and glucose improved the red pigment production on solid media.

III. NATURE OF THE AGENT PRODUCING THE METALLIC EFFECT

Giant colonies similar to those described by Hadley on Hartley's agar confirmed his findings that the metallic strains were much faster growing and more heavily pigmented than those without the metallic effect. Giant colonies of the pigmented metallic strains incubated at 37° C. reached a size of 3 cm. or more in 10 days. Those of the non-metallic yellow fluorescent strains never exceeded 1 cm. after 10 days.

Growth on different media has already been described. The metallic effect does not appear on some media, especially MacConkey's and S.S., but metallic 'plaques' reappear on subculture on Hartley's agar. Both pigment production and metallic effect were decreased on media containing excess phosphate. Use of too thin a layer of medium also inhibited the metallic effect. Both pigment production and metallic effects were present at any pH between 6·4 and 8·2, the best and most constant results occurring at approximately 7. The metallic effect showed best under aerobic conditions, and was reduced but not abolished on growth in CO_2 or under anaerobic conditions, perhaps because of the reduced rate of growth. Perhaps for the same reason pigment and metallic effect developed more slowly in cultures left at room temperature than in those at 37° C.

To try the effect of dilution of the culture on metallic 'plaque' formation, 0·1 ml. of a broth culture diluted from 10^{-1} to 10^{-7} was plated out. In the less overgrown plates, about a third of the colonies showed metallic 'plaques' after 24 hr. at 37° C., and about half the colonies after 48 hr. Sub-cultures from both metallic and non-metallic colonies, unless these were non-pigment-producing strains, showed similar growth, with metallic effect.

Agar plates, flooded with an actively growing culture and then incubated at 37° C., showed metallic 'plaques' covering the whole plate, provided that the plate had not been overdried. If plates were overdried before incubation, then only isolated metallic 'plaques' developed.

If a culture showing well-marked metallic effect was flooded with saline or distilled water, the 'metallic' film was lifted off the culture and floated on the fluid and could thus be removed. The depressed 'plaques' were left non-metallic but otherwise intact, and resembled true bacteriophage plaques. Once removed in this way the film did not appear to re-form.

To test whether *pyocyanin* itself had any influence in the production of the metallic effect a chloroform extract was made from a pigment-producing strain. The extract was dried by evaporating the chloroform and dissolved in water. This solution was 'spotted' on Hartley's agar-plate cultures of the various strains. It did not appear to have any enhancing or suppressing influence on the production of the metallic effect.

(*a*) *Effect of streptomycin*

All the strains were penicillin insensitive, and penicillin did not appear to have any effect on the metallic phenomenon. On the other hand, some strains were insensitive to streptomycin while others showed varying degrees of sensitivity. Sensitivity was determined by measuring the zone of inhibition round a small disk of filter paper soaked in streptomycin (1000 units/ml.) and placed on a Hartley's agar-plate culture of the organism. The diameter of the zones of inhibition varied up to 1·5 cm. The metallic effect in some strains reached the edge of the inhibited zone, whereas in others it stopped short, so that the inhibited zone was surrounded by a green 'halo' of normal growth, with the metallic effect beyond. Subcultures from the green 'halo' were permanently non-metallic or became so after very few subcultures.

Sulphonamides did not appear to affect the metallic appearance, though they inhibited growth of the bacteria.

(*b*) *Isolation of non-metallic variants from metallic strains*

Of separate colonies, any number from a few to 75% or more might show the metallic effect. Attempts to isolate non-metallic variants by selecting non-metallic pyocyanin-producing colonies were at first unsuccessful, whether the cultures were grown at room temperature or 37° C. After several subcultures, some colonies were non-pigmented, and subcultures from them yielded a non-metallic, usually non-pigmented variant. This seemed to occur more readily with our strains than other investigators report, for non-metallic variants were obtained after

usually less than 10 subcultures, instead of after 20–30 as reported. Metallic strains could still be recovered from the original cultures, and in several cases both variants were available. Even 6-month-old cultures would still yield viable subcultures, with good pigment and metallic 'plaque' production.

Isolation of non-metallic variants was, as already stated, simplest from streptomycin-sensitive strains, such variants still producing pyocyanin.

These non-metallic variants usually bred true, but sometimes reverted back to the metallic form after several subcultures, especially with the red strain, non-metallic non-pigmented variants of which bred true for several generations, and then developed variants which were pyocyanin producers and strongly metallic.

(*c*) *Production of metallic from non-metallic strains*

Metallic strains often arose spontaneously from non-metallic ones. Certain culture media, such as the synthetic medium already mentioned, or milk, appeared to enhance this change. Some previously non-metallic strains, both those producing pyocyanin or fluorescin and even some apparently colourless strains, would suddenly develop metallic 'plaques' and retain this property on subculturing. The original non-metallic strain could still be recovered from old stock cultures.

The first sign of the metallic change was usually a fine, almost pin-point iridescent stippling over the culture, without apparent pitting, but pitting developed after further subculturing.

(*d*) *Nomenclature adopted*

To denote the different variants isolated, a special nomenclature was introduced. The parent strains had been labelled alphabetically A, B, C to Z, and AA, AB, AC, etc. The metallic strains were further specified by the Roman numeral 'I', the non-metallic but still green, red and purple pigmented strains by the numeral 'II', and the non-metallic, yellow or non-pigmented strains by 'III'. There may therefore be three A variants, named AI, AII and AIII.

IV. ISOLATION OF *PSEUDOMONAS* BACTERIOPHAGES

It appeared likely that the metallic agent was a bacteriophage, and that the metallic effect was simply due to the deposition of a substance, derived perhaps from pyocyanin. Isolation of phages from all strains available was accordingly attempted. The method employed was similar to that used for the isolation of *Staphylococcus* phage (Fisk, 1942; Wilson & Atkinson, 1945).

One or two 6 in. Hartley's agar plates, which had been dried for 4 hr. were flooded with an actively growing 6 hr. broth culture of each of the *Pseudomonas* strains, and the excess inoculum was immediately drained off. Where available, both metallic and non-metallic variants were used as the primary inocula.

A loopful of a young broth culture of each strain—the secondary inoculum—was then placed on a marked segment of each plate or pair of plates. All plates were examined after 24 and 48 hr. incubation at 37° C.

The results were not constant, but after several attempts it could be concluded

that the metallic effect was neither inhibited nor enhanced in any combination. Thus a secondary inoculum of a non-metallic variant, while obscuring the underlying primary growth, produced neither inhibition nor increase of the metallic appearance in the surrounding primary growth. Occasional combinations showed the presence of a very faint halo of partial clearing in the immediate vicinity of the secondary inoculum.

Overnight broth cultures of each green and of the red strain were centrifuged, and the supernatant fluid heated to 60° C. for 30 min. The heated supernatant fluids were then 'spotted' on primary plate cultures of each of the non-metallic variants and produced phage plaques, none of which was metallic in appearance. Non-metallic variants were used, since the metallic 'plaques' often obscured a true bacteriophage effect.

Subsequent experiments showed that the titre of many *Pseudomonas* phage preparations was reduced by heating to 60° C. for 30 min. Broth cultures of each variant, metallic and non-metallic where available, of every strain, were therefore Seitz filtered, and the filtrates 'spotted' on plate cultures of each non-metallic variant. After incubation the site of secondary inoculation in some cases showed clearing with a few phage-resistant colonies. In others it showed isolated plaques, varying in numbers from two or three to several hundred. More difficult to interpret was partial clearance over the whole area of the second inoculum, leaving a continuous film of growth thinner than that of the adjacent primary inoculum.

The culture filtrate tests are recorded in Table 2. Combinations from which potent phage preparations were prepared for further tests are indicated.

In the tables, filtrates from metallic and non-metallic variants are shown as one, since they gave practically the same results, the metallic effect apparently not influencing the lysogenic power.

Most strains which were metallic when first isolated, appeared to be more active in their bacteriophage reactions, both in sensitivity and in lysogenic power, than those originally yellow or colourless. The originally metallic strains were the green pigment producers, A, B, C, F, G, H, K, N, P, V, X, Z, AD, AF, and AH; the purple pigment producer AB. The red strain L was non-metallic at first, but a metallic variant was isolated on subculture. It will be noted that this strain appeared to carry a latent phage active against itself.

Bacteriophage preparations were made from several of the lytic areas by sub-inoculating from them into actively growing 2 hr. broth cultures of the strains used as primary inocula. These were then further concentrated by two daily sub-inoculations of a loopful into fresh 2 hr. broth cultures. Even the last culture, containing a good deal of phage (1×10^9 phage particles/ml. or more), did not as a rule show any marked clearing when compared with a control culture. The final cultures were Seitz filtered, and the filtrates stored in the refrigerator.

The concentrated phage preparations were titrated. A loopful of each tenfold dilution was placed on a marked area of an agar plate which had been flooded with a 6 hr. broth culture of the susceptible strain. The highest dilution showing confluent lysis, usually 10^{-3} or 10^{-4}, was taken as the test dose (C.T.D.). Plaques were usually still visible in the 10^{-6} to 10^{-7} dilutions.

Table 2. *The presence of bacteriophage in culture filtrates of* Pseudomonas aeruginosa *strains*

Cultures	Filtrates																												
	A	B	C	D	E	F	G	H	K	L	N	O	P	Q	R	S	T	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH
A	−	±*	−	−	−	±*	−	−	−	(+)	−	−	−	−	−	−	−	−	(+)	−	−	−	−	−	−	−	±	+	+	−
B	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+*	−	−	−	−	+	−
C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±*	−	−	−	−	−	−
D	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
E	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
F	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±*	−	±	−	−	−	−
G	−	+*	±*	−	−	−	±*	−	−	(+)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−
H	−	+*	±*	−	−	±*	±	−	−	(+)	−	−	−	−	−	−	−	−	−	−	−	±*	−	(±)	−	−	−	+	+	−
K	−	±*	−	−	−	−	+*	−	−	±	−	−	(+)	−	±	−	−	±*	−	(+)	−	−	−	−	−	−	±	+	−	−
L	−	−	−	−	−	−	−	−	−	(+)	−	−	(+)	−	−	−	−	(+)	(+)	−	−	−	−	(+)	±	−	+	±	+	−
N	−	−	−	−	−	−	−	−	±	(+)	−	−	(+)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
O	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
P	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	+	+	−	−
Q	−	+	−	−	−	−	±	+	−		−	−	−	−	−	−	−	−	−	−	−	+	±	(+)	−	−	−	±	−	−
R	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±*	−	−	−	−	−	−
S	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±*	−	±*	−	−	−	−	−	−
T	+*	−	−	−	−	±	−	−	−	(+)	−	−	(+)	−	−	−	−	(+)	(+)	−	−	−	−	−	−	−	−	−	−	−
V	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	+	−	−
W	±	±*	−	−	−	±*	±	±	(+)	±	+*	−	(+)	−	(+)	−	−	−	−	±*	−	−	−	(+)	−	−	+	−	+	−
X	−	−	−	−	−	−	−	−	±	−	−	−	−	−	±	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−
Y	−	−	−	−	−	±*	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Z	−		Metallic			−	−	−	(+)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
AA	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
AB	−	±*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
AC	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	±	±	+	−	−	−
AD	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
AE	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	±	−	±	+	−
AF	−	−	−	−	−	−	−	−	−	−	+	−	±	−	−	+	−	−	−	−	−	+	−	−	−	±	−	−	−	−
AG	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
AH	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	+	±	±	−

*=attempts to concentrate phage failed. ( )=attempts to concentrate phage successful. ±=results variable in repeated experiments.

During these tests, it became obvious that results varied with slight differences in the medium or technique. Different batches of the same medium might give different results. Too dry a medium, too thin a layer, and excess of phosphates all inhibited phage activity. The best medium appeared to be veal infusion with 1 % agar. Phage activity was best seen on plates incubated for 16–18 hr. at 37° C.

Table 3. *Sensitivity of* Pseudomonas *strains to concentrated phage preparations*

	Phages (C.T.D.)																							
Cultures	L/A	W/A	L/G	L/H	AB/H	P/K	X/K	L/L	P/L	V/L	W/L	AB/L	L/N	P/N	AB/Q	L/T	P/T	V/T	W/T	K/W	P/W	R/W	V/W	AB/W	K/Z
AI (0)	4	–	2	4	6	–	–	5	–	–	–	5	–	3	–	–	–	2	–	5	5	4	4	4	–
AII	5	5	2	5	5	–	–	–	–	–	2	5	–	–	–	–	–	–	–	5	5	5	5	3	1
BI (0)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CI (0)	–	–	–	–	–	–	–	–	–	–	–	PC	–	–	–	–	–	–	–	–	–	PC	–	–	–
CII	–	–	–	–	–	–	–	–	–	–	–	4	–	–	2	–	–	–	–	–	–	–	–	3	–
DI	–	–	–	–	–	3	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
DII (0)	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
EI	–	–	–	–	–	PC	–	–	–	–	–	5	–	–	3	–	–	–	–	–	–	–	–	3	–
EII (0)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–
FI (0)	–	–	–	–	3	–	–	–	–	–	–	5	–	–	5	–	–	–	–	–	–	–	–	5	–
FII	–	–	–	–	3	–	–	–	–	–	–	5	–	3	4	–	–	–	–	–	–	–	–	3	–
GI (0)	6	–	2	–	3	–	–	–	–	–	2	5	–	–	5	–	1	1	–	4	3	–	2	3	–
GII	–	–	2	–	PC	–	–	–	–	–	2	5	–	–	5	–	–	2	–	5	4	PC	4	5	–
HI (0)	5	5	2	5	6	–	–	3	–	–	2	6	–	3	–	–	1	2	–	5	5	4	5	4	–
HII	6	6	6	6	6	–	–	2	–	–	2	5	1	1	–	1	–	1	1	5	6	4	5	5	–
KI (0)	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
KII	–	–	–	–	–	4	3	–	–	–	–	4	4	–	–	–	–	2	–	2	–	–	–	–	–
L series	See Table 7																								
NI (0)	–	–	–	–	–	–	3	–	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–
NII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
OII (0)	–	–	–	–	–	–	–	–	–	–	–	PC	–	–	4	–	–	–	–	3	–	–	–	–	–
PI (0)	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
QII (0)	–	2	6	2	–	–	–	–	–	2	–	5	–	–	5	–	–	–	–	5	–	–	–	5	3
RI	–	–	–	–	–	3	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	2	–	–	–
RII (0)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SII (0)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TI	2	1	–	4	3	PC	–	5	5	5	5	3	5	6	–	5	6	6	6	2	3	1	1	1	3
TII (0)	2	1	–	3	2	3	–	5	6	6	5	3	4	6	–	6	6	–	5	2	3	1	1	1	3
VI (0)	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
VII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
WII (0)	1	2	–	–	5	–	1	–	–	–	3	5	5	4	5	1	–	3	–	5	6	6	6	5	5
XI (0)	–	–	–	–	–	PC	–	–	–	–	–	–	PC	PC	–	–	–	–	–	–	–	–	–	–	–
YII (0)	–	–	–	–	3	2	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–
ZI (0)	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5
AAII (0)	–	–	–	–	2	2	1	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–
ABI (0)*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ABII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ACII (0)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
ADI (0)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AEII (0)	–	–	1	–	3	PC	–	–	–	–	–	6	5	2	5	–	–	–	–	–	5	PC	3	6	1
AFI (0)	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	3	3	3	–	3
AGII (0)	–	–	1	–	1	–	–	1	1	1	–	–	–	–	–	–	2	1	–	–	–	–	–	–	–
AHI (0)	5	5	6	4	PC	–	–	2	1	–	1	5	–	1	5	1	1	–	2	5	6	3	5	4	1

* ABI is the purple pigment-producing strain.
(0) = strain originally isolated.
L/A indicates that phage in filtrate of strain L was propagated on the susceptible strain A.
6 = complete clearing.
5 = almost complete clearing with a fair amount of resistant growth in the cleared area.
4 = confluent plaques.
3 = very numerous plaques, but with normal growth between them.
2 = up to 50 plaques per area.
1 = up to 10 plaques per area.
PC = partial clearing, the phage-inoculated area being more transparent, but still covered with a thin continuous layer of growth.
– = no evidence of phage activity.

(*a*) *Preparation and testing of bacteriophage*

Twenty-five phage preparations were tested at the test dose concentration (C.T.D.) against all *Pseudomonas* strains and variants available. The results, recorded in Table 3, indicate that there is widely divergent sensitivity to the phages.

Strains most sensitive to phages include AI, AII, HI, HII, TI, TII and AHI. On the other hand, BI, BII, NII, RII, SII, ABI, ABII and ADI were insensitive under the conditions of the test. Between these extremes the individual strains show different sensitivity spectra. Some of the more sensitive strains (AH and T) have in previous tests been found to be non-lysogenic, whereas those apparently insensitive to the concentrated phages were lysogenic (Table 2).

(*b*) *Plaque morphology*

The phage plaques varied in appearance, but each phage produced its own particular kind of plaque, irrespective of the strain on which it had been propagated. Thus some phages produced simple punched-out plaques, varying in size from a pinpoint to 0·5 mm. Others produced plaques of about 2 mm. with a central papilla of growth, surrounded by a clear ring; others, again, a similar but larger plaque with a central lytic spot in the papilla. Still others showed a halo round the plaque, sometimes quite narrow, but sometimes 4–5 mm. wide. Some plaques were surrounded by a very narrow but definite band of precipitate.

V. COMPARISON OF BACTERIOPHAGE AND METALLIC EFFECT

The true phages produce plaques which differ from those of the metallic lytic agent only in the absence of metallic sheen. The phage plaques, however, did not increase in size after 18–20 hr. incubation, and might in fact become obscured by secondary growth. The metallic plaques spread, and might even become confluent after 2–3 days, whether at 37° C. or at room temperature.

The influence of phage-containing filtrates on the metallic effect varied, not with the phage concerned, but with the metallic culture. Phage preparations never produced any metallic lesions on non-metallic strains.

The thin film of secondary growth in an area of confluent lysis produced by a potent phage filtrate acting on a 'metallic' strain was itself sometimes not metallic, but appeared clear green against the more lightly pigmented 'metallic' background, and remained so for at least 72 hr. Resistant colonies of strains which were originally less metallic, however, were sometimes more metallic in appearance than the surrounding unlysed growth.

Some variants of the red strain produced no metallic effect in lysed areas, but the pigment around the cleared area became deeper in colour and the metallic sheen became intensified about 1 cm. round the area. This was especially noticeable on plates on which the normal growth was only slightly 'metallic'.

On MacConkey's medium development of phage plaques was normal, but the metallic effect was inhibited, though not permanently. Streptomycin did not affect phage action, whereas it often inhibited the metallic effect, usually permanently. From this the metallic effect seems to be a property of bacterial variants which are more sensitive to streptomycin than are the non-metallic variants. Judging by the phage resistants seen in areas of confluent lysis, the insensitive variants were non-metallic in some cultures and metallic in others.

It appears, therefore, that the production of metallic 'plaques', pigment

production, lysogenicity, phage sensitivity and phage resistance are independent variables. These different variants were isolated from a single strain, the red strain L, in experiments reported in the next section.

VI. VARIABILITY AND BACTERIOPHAGES OF THE RED STRAIN L

Strain L was isolated on 1 July 1948 from the urine of a patient suffering from cystitis following partial resection of the bladder.

When first isolated, it resembled morphologically the other strains of *Ps. aeruginosa*, with similar rough fimbriated colonies on Hartley's agar and a yellow fluorescent pigment. After a few days at 37° C. or at room temperature the pigment changed to a clear deep red, which persisted for several months.

Table 4

Variant	Pigment	Metallic effect	Colony form	Origin
LIII–1	Blue-green	+	Fimbriated	LIII
LIII–1a	Yellow	–	Fimbriated	LIII–1
LIII–1b	Dark green	+	Fimbriated	LIII–1
LIII–1aa	Yellow	–	Fimbriated	LIII–1a
LIII–1ab	Blue-green	+	Fimbriated	LIII–1a
LIII–1bR	Dark green	+	Fimbriated	LIII–1b (phage)
LIII–2	Yellow	–	Fimbriated	LIII
LIII–2Rx	Yellow	–	Compact	LIII–2 (phage)
LIII–2Ry	Blue-green	+	Fimbriated	LIII–2 (phage)
LIII–3	Yellow	–	Compact	LIII
LIII–3a	Yellow-green	–	Compact	LIII–3
LIII–3b	Yellow	–	Fimbriated	LIII–3
LIII–3aR	Green	–	Fimbriated	LIII–3a (phage)
LIII–3bi	Yellow-green	–	Compact	LIII–3b
LIII–3bii	Yellow	–	Fimbriated	LIII–3b
LIII–3bix	Yellow	–	Compact	LIII–3bi
LIII–3biy	Green	+	Fimbriated	LIII–3bi
LIII–3biRx	None	–	Compact	LIII–3bi (phage)
LIII–3biRy	Green	+	Fimbriated	LIII–3bi (phage)
LIII–3biiRx	Yellow	–	Compact	LIII–3bii (phage)
LIII–3biiRy	Green	+	Fimbriated	LIII–3bii (phage)

When first isolated the strain was non-metallic, but a metallic variant was isolated by passage through the synthetic medium previously mentioned. Both the metallic variant, LI, and a non-metallic, LII, were therefore available for investigation. The metallic effect was of the same nature as that seen in the green strains. Some difficulty was experienced in keeping the LII variant non-metallic, even by repeated single-colony isolations, but a permanently non-metallic strain was finally obtained by subcultivation from the zone of partial inhibition produced by streptomycin.

Growth of the L strain on various solid and fluid media has already been described. The red pigment, pyorubin, was insoluble in chloroform, ether and acetone, but soluble in water and alcohol.

A third spontaneous colourless variant (LIII) produced on Hartley's agar three types of colony:

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LIII–1: a very deep blue-green colony, fimbriated and rough, showing well marked metallic effect;

LIII–2: a pale yellow, rough and fimbriated non-metallic colony; and

LIII–3: a pale yellow, almost colourless, smooth, round, compact colony, also non-metallic.

The LIII–1, 2 and 3 variants proved to be very unstable, and were constantly producing fresh variants of the same three cultural types. Further variants were obtained by routine plating or from stock cultures which had lost colour, or the reverse, or as a result of phage action. Phage-resistant (R) variants were isolated from areas of confluent lysis on plates used for testing phage filtrates.

Twenty-one variants were thus isolated from the three original LIII variants. Table 4 shows the order of their production and their chief cultural characters. Of the two colony types, the dull granular spreading one is called 'fimbriated', and the smooth round one 'compact'. The origin, identification numbers and cultural characters of all the variants are shown.

All these variants were motile, and their biochemical reactions were those of the *Pseudomonas* genus, but there was a definite tendency for the variants isolated by phage action, the resistant or 'R' strains, to be slower in their fermentation of dextrose and in other enzyme reactions.

Table 5. *Lysogenicity and sensitivity table of variants of strain L*

Cultures	Filtrates: LI	LII	LIII	LIII–1	LIII–1a	LIII–1aa	LIII–1ab	LIII–1b	LIII–1bR	LIII–2	LIII–2Rx	LIII–2Ry	LIII–3	LIII–3a	LIII–3aR	LIII–3b	LIII–3bi	LIII–3bix	LIII–3biy	LIII–3biRx	LIII–3biRy	LIII–3bii	LIII–3biiRx	LIII–3biiRy
LI	−	−	+	−	−	+	+	+	+	+	+	+	−	+	−	−	+	+	+	+	+	+	+	−
LII	±	±	+	−	−	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+
LIII	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIII–1	±	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIII–1a	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIII–1aa	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIII–1ab	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIII–1b	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	±	−	+	+	+
LIII–1bR	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIII–2	±	±	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	±	−	+	+	+
LIII–2Rx	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIII–2Ry	+	+	−	−	−	−	−	−	−	−	−	−	−	±	+	−	−	−	−	−	−	+	+	+
LIII–3	+	+	−	+	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	+	+	+	+	+
LIII–3a	+	+	−	±	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	+	+	+	+	+
LIII–3aR	−	−	−	−	−	−	−	−	±	−	+	−	−	−	−	−	−	−	−	+	+	−	−	−
LIII–3b	+	+	−	+	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	+	+	+	+	+
LIII–3bi	+	+	−	+	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	+	+	+	+	+
LIII–3biy	+	+	−	+	−	−	−	+	+	+	+	+	−	−	+	−	−	−	−	+	+	+	+	+
LIII–3biRx	±	±	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	+	+
LIII–3biRy	±	±	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	+	+
LIII–3bii	±	±	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	−	−	−	±	−
LIII–3biiRx	−	−	−	+	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−
LIII–3biiRy	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−

+ = definite phage activity on several occasions. ± = variable results in repeated tests.

Bacteriophage activity of variants of strain L

Latent phages could be shown easily in cultures of many of the *Pseudomonas* strains by testing filtrates of broth cultures of each strain against all the organisms. The L strain was unusual in that it carried a phage or phages which were shown in several tests to be active against its own variants.

Filtrates of broth cultures of each variant were therefore tested against the other variant and against itself. Table 5 shows that there was considerable variation in phage activity, independently of colony appearances, pigment production, or the metallic effect. Several green metallic strains gave very similar results, both as regards latent phages and sensitivity, to those of yellow non-metallic strains, such as LIII-1bR, and LIII-2Rx, LIII-2 and LIII-2Ry.

The sensitivity of the original LI and LII strains was striking, since these were sensitive to most of the phages, including some which acted on none of the other variants.

By grouping together those with similar phage sensitivities and lysogenic powers the LIII variants could be divided into the following ten groups:

Group		
A	LIII	= colourless, non-metallic, compact
	LIII–1aa	= yellow, non-metallic, fimbriated
	LIII–1ab	= green, metallic, fimbriated
B	LIII–1	= blue-green, metallic, fimbriated
C	LIII–1a	= yellow, non-metallic, fimbriated
D	LIII–1b	= green, metallic, fimbriated, later yellow, non-metallic, fimbriated
	LIII–2	= yellow, non-metallic, fimbriated
	LIII–2Ry	= green, metallic, fimbriated
E	LIII–1bR	= green, metallic, fimbriated
	LIII–2Rx	= yellow, non-metallic, fimbriated
F	LIII–3	= yellow, non-metallic, compact
	LIII–3b	= yellow, non-metallic, fimbriated
G	LIII–3a	= yellow, non-metallic, compact
	LIII–3bi	= yellow, non-metallic, compact
	LIII–3bix	= colourless, non-metallic, compact
	LIII–3biy	= green, metallic, fimbriated
H	LIII–3aR	= green, non-metallic, fimbriated
K	LIII–3biRx	= colourless, non-metallic, compact
	LIII–3biRy	= green, metallic, fimbriated
	LIII–3bii	= yellow, non-metallic, fimbriated (All these inconstant in reactions)
M	LIII–3biiRx	= yellow, non-metallic compact
	LIII–3biiRy	= green, non-metallic, fimbriated

The phages, on the other hand, can be subdivided into the following groups:

Group 1: Carried by LI and LII, active against all variants, except those from LIII–1a and most 'R' strains.

Group 2: Carried by LIII–1, or group B, active only against the most sensitive of the LIII–3 variants.

Group 3: Carried by LIII–1aa, lab, 3a, and 3bi, 3bix and 3biy, groups A and G, active only against LI and LII.

Group 4: Carried by 1b, 2 and 2Ry, i.e. group D, active against LI, LII, the sensitive LIII–3 strains, but not against LIII–1 and 2 derivatives, and only the 3bii resistant 'R' strains.

Group 5: Carried by 1bR and 2Rx, i.e. the E group, the resistant strains of the previous group, active against the same variants, but including 3aR.

Group 6: That of 3aR, group H and 3biiR strains, or M group, active against all sensitive and 3biR strains.

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Table 6. *Susceptibility of* Pseudomonas *'L' variants to phages in filtrates of other* Pseudomonas aeruginosa *cultures*

	Filtrates																											
Cultures	A	B	C	D	E	F	G	H	K	L	N	O	P	Q	R	S	T	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH
LI	–	–	–	–	–	–	5	3	2	3	–	–	–	–	3	–	–	2	–*	–	–	–	–	5	3	–	5	5	5	–
LII	PC	PC	PC	PC	–	–	4	–	2	–	–	–	–	–	3	–	–	–	–	–	–	–	–	5	3	–	5	5	5	3
LIII	3	PC	–	–	–	–	–	3	2	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	2	–	5	2	–	–
LIII-1	4	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	PC	–	–	2	–	PC	–	–	–
LIII-1a	4	PC	–	–	–	–	5	4	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	2	–	5	5	–	–
LIII-1aa	3	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	PC	–	–	–	–	–	–	–	–
LIII-1ab	3	–	–	–	–	–	–	3	–	3	–	–	–	–	–	–	–	–	–	–	–	5	–	–	1	–	PC	PC	–	–
LIII-1b	PC	–	–	–	–	–	PC	3	2	1	–	–	4	–	–	–	–	3	–	–	–	4	–	–	2	–	4	–	–	–
LIII-1bR	4	–	–	–	–	–	PC	3	2	1	–	–	–	–	–	–	–	3	–	–	–	PC	–	–	2	–	5	PC	–	–
LIII-2	4	–	–	–	–	–	–	3	2	1	–	–	4	–	–	–	–	3	–	–	–	PC	–	–	1	–	5	–	–	–
LIII-2Rx	PC	–	–	–	–	–	PC	PC	–	–	–	–	–	–	–	–	–	–	–	–	–	5	–	–	–	–	5	5	–	–
LIII-2Ry	4	–	–	–	–	–	–	3	3	2	–	–	PC	–	–	–	–	PC	–	–	–	–	–	–	2	–	5	PC	3	–
LIII-3	–	–	–	–	–	–	–	–	–	1	–	–	4	–	–	–	–	4	–	–	–	1	–	–	1	–	5	3	–	–
LIII-3a	–	5	5	–	–	–	–	–	–	–	–	–	4	–	–	–	–	–	–	–	–	5	–	–	–	–	5	5	–	–
LIII-3aR	–	PC	–	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	PC	–	–	–	–	–	5	–	–
LIII-3b	1	PC	PC	–	–	–	5	–	–	–	–	–	5	–	–	–	–	3	–	–	–	–	–	–	2	–	3	PC	1	–
LIII-3bi	1	–	–	–	–	–	5	3	–	1	–	–	5	–	1	–	–	4	–	–	–	2	–	–	2	–	4	5	3	–
LIII-3bix	1	PC	–	–	–	–	5	–	–	1	–	–	5	–	3	–	–	3	–	–	–	5	–	4	3	–	3	4	4	–
LIII-3biy	–	–	PC	–	–	–	5	1	1	1	–	–	4	–	2	–	–	4	–	–	–	5	–	4	2	–	5	5	4	–
LIII-3biRx	–	4	4	–	–	–	5	–	–	–	–	–	5	–	–	–	–	3	–	–	–	5	–	–	–	–	5	6	–	–
LIII-3biRy	–	–	–	–	–	–	5	–	–	1	–	–	5	–	1	–	–	2	–	–	–	5	–	–	2	–	5	4	–	–
LIII-3bii	4	–	–	–	–	–	–	4	3	1	–	–	5	–	2	–	–	3	–	–	–	2	–	–	2	–	4	–	3	–
LIII-3biiRx	–	4	4	–	–	–	6	–	–	–	–	–	5	–	3	–	–	4	–	–	–	5	–	3	1	–	5	6	3	–
LIII-3biiRy	–	–	4	–	–	–	5	1	–	–	–	–	PC	–	2	–	–	4	–	–	–	5	–	–	2	–	5	5	2	–

* Variable results obtained (see Table 2).

Group 7: Carried by LIII–3biR, and 3bii, the K group, with rather variable action on all sensitive strains, and also on 3aR and 3biiR strains.

No phages appear to be carried by LIII–1a, LIII–3, or LIII–3b.

These phages all produced similar plaques, suggesting that they may be variants of one phage rather than of different types of phage. Individual plaques varied in size from a pinpoint to $\frac{1}{2}$ mm. in diameter, with slightly hazy irregular edges.

The L variants were also tested for their sensitivity to phages in filtrates of the other strains of *Ps. aeruginosa*. The results are summarized in Table 6. Here, too, the variants showed differences in sensitivity. It is likely that at least some of the phages in these filtrates were of different types rather than variants of the same type; their plaques showed striking differences.

Table 7. *Sensitivity of variants of L strain of* Pseudomonas aeruginosa *to concentrated phage preparations*

	Phages (C.T.D.)																							
Cultures	L A	W A	L G	L H	AB H	P K	X K	L L	P L	V L	W L	AB L	L N	P N	AB Q	L T	P T	V T	W T	K W	P W	R W	V W	AB W	K Z
LI	–	–	3	–	4	–	–	–	–	–	–	5	5	–	5	–	–	–	–	5	6	5	5	4	1
LII	–	–	PC	–	–	–	–	–	–	–	–	5	–	–	5	–	–	–	–	5	6	5	6	5	–
LIII	–	–	–	2	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LIII-1	–	–	–	1	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LIII-1a	–	–	–	1	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LIII-1aa	–	–	–	1	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LIII-1ab	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LIII-1b	3	3	–	2	3	5	–	1	–	–	5	4	5	–	–	5	–	1	5	1	2	1	1	1	2
LIII-1bR	–	–	–	1	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LIII-2	4	4	–	3	4	5	–	1	–	–	5	4	6	3	–	5	–	1	5	4	3	2	2	2	4
LIII-2Rx	–	–	–	2	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3
LIII-2Ry	3	3	–	2	2	5	–	1	–	–	6	3	5	3	–	5	–	1	5	2	2	–	2	2	3
LIII-3	3	3	2	1	1	5	–	6	6	6	6	3	–	5	–	5	6	6	6	3	2	1	1	1	1
LIII-3a	1	1	–	–	–	–	–	5	5	5	5	2	–	–	–	5	5	5	5	–	–	–	–	–	–
LIII-3aR	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	5	5	–	–	5	3	4	–	–
LIII-3b	3	2	–	1	–	5	–	4	4	3	5	2	–	3	–	6	5	5	6	2	2	1	1	1	3
LIII-3bi	3	3	1	2	–	5	–	5	5	5	6	3	–	3	–	6	6	6	6	2	2	–	2	1	1
LIII-3bix	3	3	3	2	–	5	–	5	5	5	5	3	3	5	–	5	5	5	5	2	2	2	1	2	2
LIII-3biy	3	3	2	2	1	5	–	5	5	5	5	3	–	5	–	5	6	5	5	1	2	–	1	–	2
LIII-3biRx	2	2	–	–	1	5	–	–	–	–	5	3	–	5	–	6	–	–	5	1	1	–	1	1	2
LIII-3biRy	2	2	–	1	–	5	–	–	–	–	5	3	5	5	–	5	–	–	5	3	–	1	–	1	2
LIII-3bii	3	3	–	1	3	4	1	1	–	–	5	3	4	3	–	5	–	–	5	–	2	–	1	1	2
LIII-3biiRx	–	–	–	–	–	5	–	3	4	3	–	–	2	3	–	5	5	5	–	–	6	5	5	–	2
LIII-3biiRy	–	–	–	–	–	5	–	3	4	3	–	–	2	3	–	3	5	5	–	–	6	3	4	–	4

The phage in filtrate H produces on LIII–2 a ring-like plaque, with an apparently raised centre, and a wide surrounding zone of partial lysis 4–5 mm. wide. The phage in V filtrates produces on 3b1 a smaller ring-like plaque, with a narrow intense area of precipitate round the plaque. The intensities of the precipitate varied from time to time and apparently depended to some extent on the medium used. A third distinct plaque type was seen when filtrates of AG were plated on cultures of the strain 3b1. This was a very tiny plaque producing only a shallow superficial pitting of the growth, difficult to see with the naked eye, but occasionally made more obvious by a thin precipitate covering the whole plaque.

The results in Table 6 agree with tests for the sensitivity of L variants to L phages in allowing the L variants to be grouped according to phage activity.

The sensitivity of the L variants to a number of bacteriophage concentrates was also tested. The phage concentrates were prepared by propagating phages in the

filtrates of primary cultures on other strains susceptible to their action. The concentrates were titrated on the strain used for their propagation and the highest dilution producing confluent lysis (C.T.D.) used in testing the sensitivity of the L variants.

Table 7 shows that the activity of some phages corresponded to that of a simple filtrate of the host strain used to prepare them. Thus, phages grown on A and H gave results comparable to simple filtrates of A and H cultures, even in the appearance of the plaques. On the other hand, many of the concentrates showed on the L variants ranges of activity different from those of simple filtrates of host cultures. At least one concentrated phage, X/K, had practically no activity on any of the L variants, whereas simple filtrates of K cultures were active against at least nine of them.

VII. DISCUSSION

This investigation arose from our interest in the metallic effect so frequently seen in freshly isolated cultures of *Ps. aeruginosa*. Not only has it proved impossible to isolate any single agent responsible for these metallic iridescent 'plaques', but it has also been impossible to produce or inhibit the effect at will, except by the isolation of single colony variants.

Apart from the iridescence, the 'plaques' are typical of those resulting from bacteriophage action, and it remains possible that the effect is due to a bacteriophage, perhaps acting together with the deposition of an altered or abnormal metabolic or breakdown product of the cell. It is not unlikely, for instance, that the precipitate seen in plaques of several of the phages on susceptible non-pigmented bacterial strains may be in some way related to the material with a metallic lustre seen in plaques on active pigment producers. On the other hand, there are several points of difference between the agent causing the metallic effect and true bacteriophages. Thus, no active filtrable agent has so far been recovered which will produce the metallic effect; the metallic effect is a progressive one, extending over several days, while bacteriophage action usually is at its maximum after 24 hr.; the metallic effect is inhibited by MacConkey's and S.S. medium, on which phage action remains unaltered; the metallic effect is inhibited permanently in certain sensitive strains by streptomycin, whereas the phage activity does not appear to be affected.

The investigation was broadened to a general study of *Ps. aeruginosa* phages. A wide range of spontaneous variation occurs in the *Pseudomonas* group, particularly in the change from pigment-producing metallic strains to yellow non-metallic ones. The reverse mutation also occurs, and a non-metallic strain may develop pyocyanin-producing powers, and eventually even become metallic. A mutant of this type, growing as a rule more rapidly, soon outgrows the slower yellow strain.

This group of organisms seems to offer a fruitful field for the study of general problems of bacteriophage growth. One strain (L) has been studied in some detail. As originally isolated it appeared to consist of a mixture of three variants, LI, II and III, differing in pigment production. LIII could be further subdivided by picking single colonies from plate cultures. One of these, LIII–3, is non-lysogenic,

yet successive variants were isolated from it, especially its phage-resistant variants, which showed greater lysogenic powers. Phage must therefore have been present in the parent strain 3, in a latent form, becoming obvious only on isolation of variants allowing free multiplication of the phage. Associated with this increase in lysogenic power there was often a decrease in bacteriophage sensitivity.

The lysogenicity, phage sensitivity and colony appearances of the L strain appeared to undergo independent variations. It is tempting to speculate whether bacteriophages which are present may not be concerned in the production of the bacterial variants. Thus the variant LIII–2 appeared to be stable in colony characters through many successive subcultures, but, as a result of phage action, a variant, 2Ry, occurs which differs in pigment production and in the presence of metallic plaques. It could even be suggested that the phage had in this case induced the power to develop metallic plaques.

It may, on the other hand, be considered that both the bacteria and the bacteriophages they carry are continually undergoing mutations. The appearances of the cultures and the specificity of the phages will at any one time depend on which variants are encouraged by the particular cultural conditions and phage-host relationship existing. The resulting picture is at present complex, but may well be a fruitful field for study by bacterial geneticists and those interested in the phenomenon of lysogenicity.

Only a broad grouping of the *Pseudomonas* strains according to their sensitivity to the available phages and their lysogenicity was possible. It is apparent that a variety of bacteriophages and their variants are carried by *Ps. aeruginosa* strains. Some of these phages show differences in appearance of plaques and in host range. Many are undoubtedly variants of single-phage strains. Of particular interest is the inhibition of a phage naturally present in a culture by the propagation of phage from another filtrate or the modification of the host range by such propagation of a new phage (Table 7).

The phages no doubt differ in their ability to multiply under different environmental conditions and particularly in different host cells. Newly added phages may or may not outgrow those normally present in a lysogenic strain and thus account for the altered range of specificity seen with the phage concentrates. Consideration must, however, also be given to the possibility of conjugation between phages analogous to that described by Luria & Dulbecco (1949) for ultra-violet light-inactivated *Bacterium coli* phages.

It is intended to investigate the *Pseudomonas aeruginosa* bacteriophages and particularly those active on the L variants in greater detail.

VIII. SUMMARY

Thirty strains of *Pseudomonas aeruginosa*, isolated from routine clinical material, were investigated, with particular reference to the metallic lytic effect and the bacteriophages they carry. From the filtrates of these strains, twenty-five bacteriophages or phage variants were isolated, and the effect of these on the various bacterial strains and some of their variants were investigated.

It has not been possible to elucidate the nature of the agent responsible for the metallic lytic effect. The true phages isolated, however, present many features of interest which warrant their more detailed study.

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