Lysogeny in *Proteus rettgeri* and the Host-Range of *P. rettgeri* and *P. hauseri* Bacteriophages

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SUMMARY

Twenty-two different phage-types of *Proteus rettgeri* were investigated for lysogeny by growing strains singly, in mixtures and by ultraviolet induction. Five of the strains were lysogenic for other members of the group. The phages differed from one another in host-ranges. Eighteen different lytic phages were isolated from sewage with action on one or more of the strains. The host-range of all 23 phages was tested against a number of *P. hauseri*, *P. morganii*, Providence and other intestinal organisms. Twelve of the phages had productive cycles of infection on 24 strains of *P. hauseri*; 21 of the phages attacked 19 Providence strains. Two of the phages lysed the same 2 out of 40 strains of *Escherichia coli*. The efficiencies of plating varied from 10^{-4} to unity. No action was demonstrated on any of the *P. morganii* or other strains tested. The host-range of 23 *P. hauseri* phages previously isolated from sewage and from lysogenic *P. hauseri* was tested against *P. rettgeri*, *P. morganii* and Providence strains. Three Providence and 2 *P. morganii* strains proved susceptible to productive infection by a number of the *P. hauseri* phages; 15 *P. rettgeri* strains were lysed by one or more of 18 *P. hauseri* phages. Some of the *P. hauseri* phages also caused abortive infection associated with cell death in some *P. morganii* and *P. rettgeri* organisms. The results indicate close ties between the *P. hauseri* and *P. rettgeri* groups, and between *P. rettgeri* and the Providence strains.

INTRODUCTION

Differences of opinion exist whether organisms of the *Proteus rettgeri* and *P. morganii* groups should be included with *P. hauseri* in the genus *Proteus*. Kauffmann (1951) suggested combining *P. rettgeri* and *P. morganii* strains in one group. Topley & Wilson's *Principles* (1955) included *P. morganii* strains in the *Proteus* group but considered *P. rettgeri* strains to be more closely related to paracolon organisms and proposed the name 'Bacterium rettgeri' for them. On the basis of the guanidine + cytosine content of their deoxyribonucleic acids Falkow, Ryman & Washington (1962) showed that *P. morganii* strains were distinct from *P. hauseri*, *P. rettgeri* and Providence strains. The latter three organisms possess very similar base compositions. The taxonomic relationships of the Providence group is also uncertain. Kauffmann (1951) described Providence strains as intermediate between *Shigella* and *Proteus*. The latter author as well as Ewing, Tanner & Dennard (1954) stressed the biochemical relationships between Providence, *P. rettgeri* and *P. morganii*. Topley & Wilson's *Principles* (1955) relegated Providence strains to a subgroup of paracolon organisms. *P. hauseri* is certainly a well-defined group; it differs markedly

in its biochemical reactions from other members of the Enterobacteriaceae (Topley & Wilson, 1955) and only isolated instances of serological cross-reactions with other members of this family have been recorded (Kauffmann, 1951; Namioka & Sakazaki, 1959). Bacteriophage reactions have confirmed the above findings. Phages of *P. hauseri* were tested by Brandis & Schwarzrock (1956), Coetzee (1958) and Vieu (1958) for action on many strains of *Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Staphylococcus*, and paracolons. Vieu also tested *P. hauseri* phages against a number of *P. morganii*, *P. rettgeri* and Providence strains. Apart from the finding by Vieu that an unspecified number of *P. hauseri* phages produce 'incomplete lysis or some isolated plaques' on 2 of 18 *P. rettgeri* strains no other positive results have been recorded. In examining the haemagglutinating activity of *P. rettgeri* and *P. morganii* strains (Coetzee, Pernet & Theron, 1962) it was found that while *P. rettgeri* strains differed from *P. hauseri* and *P. morganii* in the range of species of vertebrate red cells agglutinated the haemagglutinins of all three groups shared the property of not being inhibited by mannose (Shedden, 1962). This finding and the observation by Vieu (1958) led us to investigate the bacteriophage relationships of *P. hauseri*, *P. rettgeri*, Providence and *P. morganii* organisms.

METHODS

Organisms. Twenty-eight strains of *Proteus hauseri* were used. Nine were 'vulgaris' varieties and the remainder 'mirabilis' strains. They were isolated from human faeces or urine and all but four (nos. 108, 121, 171, 192) are hosts for a series of phages (Coetzee, 1958; Coetzee & Sacks, 1960*a*). Identifying numbers are given in Table 3. All the cultures were in the A morphological phase (Coetzee & Sacks, 1960*b*) and when the swarms were matched on nutrient agar plates they all showed a narrow line of demarcation. This is taken to mean that the strains differ in some respect (Dienes, 1946; Krikler, 1953; Story, 1954). Fourteen locally isolated strains of *P. rettgeri* and 8 *P. rettgeri* cultures obtained from the National Collection of Type Cultures (NCTC; Colindale, London) were used. Identifying numbers are given in Table 1. The *P. morganii* strains used comprised 6 NCTC strains and 11 locally isolated cultures. The identifying numbers are NCTC 1707, 2815, 2818, 5845, 7381, 10041, and local no. M2, M3, M21, M47, M49, M71, M86, M92, M232, M235, M336. Twenty-four different O serological groups of Providence strains were used; they were supplied by Miss Shona Wright (Bacteriology Department, University of Edinburgh). They are all NCTC cultures and their identifying numbers are given in Table 4. Five different phage-types of *Salmonella typhosa*, 23 other *Salmonella* serological varieties, 40 different isolates of *Escherichia coli*, 8 different isolates of paracolon organisms, 8 *Shigella* strains and 7 strains of *Pseudomonas aeruginosa* were used in host-range experiments. Cultures were stored on agar slopes at 4°.

Phages. The 23 *Proteus hauseri* phages used are listed in Table 2. Twenty-one were isolated from sewage (Coetzee, 1958), the remaining two are virulent clear plaque mutants of temperate phages 34/13 and 12/57 (Coetzee & Sacks, 1960*a, c*). Twenty-three *P. rettgeri* phages were isolated during the present work from sewage and from lysogenic strains. The sewage isolations were by the enrichment technique of Adams (1959). Lysogeny was tested for by examining the supernatants of

10-day-old broth cultures of single organisms and mixtures of organisms for phage ultraviolet irradiation of cultures was also used. These three methods of induction have been described (Coetzee & Sacks, 1959, 1960*a*). All the *P. rettgeri* strains were used as possible indicators. Phages isolated from sewage are identified by the number of the original indicator organism. Temperate phages are identified by fractions: the number of the lysogenic strain is the nominator and that of the indicator strain the denominator. The *P. rettgeri* phages are listed in Table 1.

The nutrient agar and general methods used were those of Adams (1959); the broth was as described by Coetzee & Sacks (1960*b*). Phages were purified by three successive single-plaque propagations on the host organism. Broth lysates were shaken with 0.1 vol. chloroform to kill bacteria, decanted to remove excess chloroform and aerated for about 30 min. at 45° to remove residual chloroform. Lysates were stored at 4° in sterile containers. The host ranges of the phages were determined by spotting drops of phage suspensions on agar plates which had been layered with soft agar seeded with enough test organism to give a lawn. The phage suspensions contained about 10⁸ plaque-forming particles/ml. Plates were incubated at 37° overnight before reading. Phages which showed reactions were then titrated simultaneously on the original host organism and on the new strains and the relative efficiency of plating (e.o.p.) calculated. Abortive infections were investigated by mixing phage and organism at a multiplicity of infection of about 1 at 37°. After an adsorption period of 5 min. an aliquot was treated with chloroform and titrated for unadsorbed phage on the homologous host. Simultaneously another aliquot was titrated for viable bacteria and for total infective centres on both the organisms involved (Coetzee, De Klerk & Sacks, 1960). Phages which had a low e.o.p. on heterologous hosts were investigated according to the above method using the homologous and heterologous organisms in separate experiments. These experiments were then repeated with the phage prepared on the heterologous host. In these experiments total infective centres were also assayed at the end of the latent periods (Bertani & Weigle, 1953). In attempts to increase the e.o.p. or to convert abortive to productive infections the top-layer agar was enriched with 10 g. vitamin-free pancreatic digest of casein/l. (Nutritional Biochemicals Corp., Cleveland, Ohio), 0.2 M-sodium chloride and ammonium phosphate, 0.01 M-calcium chloride and 0.01 M-magnesium sulphate individually or together.

RESULTS

Demonstration of lysogeny in Proteus rettgeri. Five of the *P. rettgeri* strains were lysogenic for one or more of the 22 strains of this group; phages were obtained by all three methods used. The intra-group host-range of these phages (Table 1) shows that they can be differentiated on their host-ranges. Taubeneck (1962) did not find lysogeny in 4 *P. rettgeri* strains he examined.

Isolation of Proteus rettgeri phages from sewage. Phages were isolated from sewage against 18 of the *P. rettgeri* strains. The intra-group host-range (Table 1) shows that these phages can be distinguished on the basis of host-range and that the 22 hosts differ from one another in susceptibility to these phages. The e.o.p. of all the *P. rettgeri* phages on heterologous *P. rettgeri* hosts was unity.

Intra-group host-range of Proteus hauseri phages. The phages isolated on *Proteus*

Table 1. *Intra-group host-range of Proteus rettgeri phages*

Plaque-forming titres were determined on homologous and heterologous hosts and the relative efficiencies of plating calculated.

<i>P. rettgeri</i> phage		<i>P. rettgeri</i> strains																			
		NCTC no.						Local organisms no.													
8893	7481	7480	7479	7478	7477	7476	7475	R 400	R 327	R 326	R 325	R 322	R 300	R 88	R 67	R 65	R 51	R 34	R 13	R 12	R 6
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
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1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1					

Numbers = relative efficiency of plating. Points = no action.

Table 2. *Intra-group host-range of Proteus hauseri phages*

Plaque-forming titres were determined on homologous and heterologous hosts and the relative efficiency of plating calculated. Only phage-susceptible strains are listed.

<i>P. hauseri</i> phage	<i>P. hauseri</i> strains															
	'mirabilis'								'vulgaris'							
	6	12	13	14	19	20	23	24	25	34	57	58	63	65	74	78
6	1	1	1	1	.	1	.	1	1	1	.	1	.	.	.	.
12	.	1	.	.	.	.	.	1	1	1	.	1	.	.	.	1
13	1	1	1	.	1	1	1	.	1	1	.	1	.	.	.	1
14	1	1	1	1	1	1	1	1	1	1	.	1	.	.	.	.
19	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.
20	.	.	.	.	1	1	1	1	1	1	.	1	.	.	.	1
23	1	1	.	1	.	1	1	1	1	1	.	1	.	.	.	.
24	.	.	.	1	.	1	.	1	.	.	.	1	.	.	1	.
25	.	.	.	.	.	1	.	1	1	.	.	1	.	.	.	.
34	1	.	.	.	1	1	1	1	1	1	.	1	.	.	1	.
58	1	.	1	1	.	1	.	1	1	1	.	1	.	.	1	1
63	.	1	1	1	.	.	.	.	.	.	.	.	.	.	.	.
65	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.
74	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1	.
34/13 vir	.	.	1	.	.	.	.	.	.	.	1	.	.	.	.	.
12/57 vir	.	.	.	.	.	.	.	.	.	.	1	.	.	.	.	.
9	.	.	.	.	.	.	.	1	.	.	.	.	.	1	.	.
48	1	1	1	1	.	1	1	1	1	1	.	1	.	.	1	.
49	.	.	.	.	.	.	.	1	.	.	.	1	.	.	1	.
51	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
54	.	1	.	.	.	.	.	.	.	.	.	.	.	.	1	1
61	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	1
78	1	.	.	.	.	.	.	.	.	.	1	.	.	.	.	1

Numbers = relative efficiency of plating. Points = no action.

Table 3. *Action of Proteus rettgeri phage on strains of P. hauseri*

Plaque-forming titres were determined on homologous and heterologous hosts and the relative efficiency of plating calculated. Only phages with one or more positive reactions are listed.

phage <i>P. rettgeri</i>	<i>P. hauseri</i> strains																			
	'mirabilis'										'vulgaris'									
	6	12	13	14	19	20	23	24	25	34	57	58	63	65	74	108	121	171	192	
7481	.	.	0	.	.	.	.	-1	.	.	.	.	.	.	0	.	.	0	.	.
7477	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	.	.	-2	.	.
7475	0	0	.	.	.	.	.	-1	-1	.	.	.	.	-1	.	.	.	.	.	.
400	.	0	.	.	.	.	.	0	.	.	.	.	.	-1	.	.	.	.	.	.
325	.	0	.	.	.	.	.	.	.	.	.	.	.	.	.	-1	.	.	.	.
322	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1	.	.	.
300	.	.	.	.	.	.	.	.	.	-1	.	.	.	.	.	.	.	.	.	.
R51	.	.	.	.	0	.	.	.	.	.	0	.	.	.	.	.	.	.	.	.
R13	0	.	.	.	.	.	.	.	-1	.	.	.	.	.	.	.	.	.	.	.
7478/325	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7476/322	.	.	.	.	.	.	.	.	.	.	0	.	.	.	.	.	.	.	.	.
400/300	.	.	.	.	.	.	.	.	-1	.	-2	-2	-2	.	.	.	.	.	.	.

Numbers = log₁₀ of relative efficiency of plating. Points = no action.

Table 4. *Action of Proteus rettgeri phage on Providence strains*

Plaque-forming titres were determined on homologous and heterologous hosts and the relative efficiencies of plating determined. Only phages with one or more positive reactions are listed.

<i>P. rettgeri</i> phage	Providence strains (NCTC no.)																							
	9000	9190	9211	9213	9215	9219	9224	9246	9248	9250	9260	9262	9265	9268	9271	9276	9278	9279	9282	9283	9290	9295	9298	9402
8898	-1	.	.	0	-1	-1	.	-2	.	-1	.	.	-2	-1	.	.	.	.	.	-2	-1	.	.	0
7481	-1	.	.	-3	.	.	.	-1	-4	-1	-1	.	0	.	.	0	.	0	.	-3	.	.	.	0
7480	-1	.	.	.	-2	-1	-4	-2	.	-2	.	.	-2	-2	.	.	.	.	.	-2	.	.	.	-2
7479	0	0	-4	0	0	-1	0	.	.	0	.	.	0	0	.	.	.	.	.	0	0	.	.	0
7477	-1	.	.	-1	0	-1	.	-1	-1	-1	-1	.	0	-1	.	0	0	0	.	-1	-1	.	.	-1
7475	-3	-4	.	-1	-1	-2	.	-1	.	-3	.	.	-1	-4	.	-1	.	-1	.	.	-2	.	.	-2
R400	-2	-2	.	-1	-1	-1	.	-1	.	-1	-1	.	0	-1	.	0	.	0	.	-1	-1	.	.	-2
R326	-1	0	.	0	-2	-2	.	.	-2	-2	-1	.	0	-2	.	-1	.	-1	.	-1	-1	.	.	0
R325	-3	.	.	0	.	.	.	.	.	.	.	.	-1	.	.	-1	.	-3	.	.	.	.	.	-1
R322	-1	.	.	-2	-2	.	.	.	.	-3	.	.	-2	.	.	-1	.	-1	.	.	.	.	.	-1
R300	-3	-1	.	-1	-1	-1	.	.	.	.	.	.	-1	-1	.	.	.	-4	.	.	.	.	.	-4
R88	.	0	-1	-3	-3	.	-3	.	-3	.	-3	.	.	.	.	.	.	.	.	.	.	0	-2	-2
R67	.	.	.	0	-1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-1	-1	0
R65	.	.	.	0	0	-2	-2	.	.	.	.	.	-2	.	.	.	.	.	.	.	0	-1	.	.
R51	-1	.	-4	-3	-3	.	.	.	.	.	.	.	-3	.	.	-3	.	.	.	.	.	.	.	-3
R18	-2	0	-1	0	0	-2	0	.	-2	.	0	.	-1	-2	.	-2	.	.	.	0	0	.	.	0
R12	-4	0	-1	-4	-1	.	.	.	-2	-1	-1	.	-4	.	.	.	.	.	.	.	-1	.	.	-1
R6	.	.	.	0	-2	-1	.	.	-2	-2	-1	.	-2	-2	.	.	.	.	.	-2	0	.	.	0
R6/322	.	.	.	.	-2	.	.	.	.	-2	.	.	.	-1	.	-1	.	.	.	.	-1	.	.	.
7478/325	.	.	.	.	-3	-2	.	-2	.	-2	.	.	.	.	.	.	.	.	.	.	-1	.	.	.

Numbers = log₁₀ of relative efficiency of plating. Points = no action.

hauseri strains (Table 2) differed from one another in host-range and, as shown (Coetzee, 1958), many have the same e.o.p. on *P. mirabilis* and *vulgaris* varieties of *P. hauseri*.

Action of Proteus rettgeri phages on P. hauseri organisms. Table 3 shows that 12 of the *P. rettgeri* phages have productive cycles on several *P. hauseri* hosts. The e.o.p. was often unity. A few of the systems with low e.o.p. were further examined. A typical example is the action of *P. rettgeri* phage 7477 on *P. mirabilis* strain 192. This phage has an e.o.p. of slightly less than 10^{-2} on the 'mirabilis' strain, adsorbs equally well on both the organisms but kills few of the 'mirabilis' organisms. Phage prepared on strain 192 plates with equal efficiency on the two strains. However, a single cycle of growth in *P. rettgeri* 7477 is sufficient for it to revert to the original state with an e.o.p. of about 10^{-2} on *P. mirabilis* 192. The efficiency of this transformation makes an explanation on grounds of host-range mutants untenable (Bertani & Weigle, 1953) and suggests a phenotypic modification of the phage by the *P. rettgeri* strain (Luria, 1953). Addition of casein digest or the various salts to the top-layer agar did not affect the e.o.p. The phenomenon was not further investigated.

Table 5. *Action of Proteus hauseri phage on P. rettgeri strains*

Plaque-forming titres determined on homologous and heterologous hosts and relative efficiencies of plating calculated. Only systems showing one or more positive reactions are presented.

<i>P. hauseri</i> phage	<i>P. rettgeri</i> strains														
	NCTC 7481	NCTC 7479	R400	R327	R326	R325	R322	R300	R83	R67	R51	R34	R13	R12	R6
6	-1	.	.	.	-1	.	.	.	.	.	.	.	.	.	0
12	-1	.	.	.	-1	.	A	.	-3	.	.	A	A	.	.
13	-1	.	.	.	-1	.	.	.	.	.	-1	.	-1	.	.
14	-1	.	.	.	-1	-3	.	.	-3	.	.	.	.	A	.
19	-1	.	.	.	-1	.	.	-2	.	.	.	.	.	.	.
20	-1	.	-3	.	-1	A	.	.	.	-2	.	.	.	.	.
23	-1	.	.	.	-1	.	A	.	.	.	.	.	-2	.	.
24	-1	.	.	-2	-1	.	.	.	-2	.	.	-3	.	A	.
25	-1	.	.	.	-1	.	-3	-3	A	.	.	.	.	.	-1
34	-1	.	.	.	-1	.	.	.	.	.	.	-1	.	.	.
58	-1	.	.	.	-1	A	.	.	.	.	.	.	.	A	.
63	-1	.	.	-3	-1	.	.	.	.	.	-1	.	-3	.	.
74	-1	.	.	.	-1	.	0	.	-2	.	.	.	.	.	-1
34/13 vir	-1	-1	.	.	.	A	.	.	.	.	.	.	.	.	.
9	-1	.	.	.	-1	.	.	.	.	-1	.	.	.	-2	.
48	-1	.	.	.	-1	.	.	.	.	.	A	.	.	.	.
54	-1	.	-2	.	-1	.	0	.	.	.	.	.	.	.	.
61	-1	.	.	.	-1	.	.	.	.	.	A	.	.	A	.

Numbers = $\log_{10}$ of relative efficiency of plating. A = abortive infection. Points = no action.

Action of Proteus rettgeri phage on P. morganii organisms. No action of any kind was observed for *P. rettgeri* phages on the 17 *P. morganii* strains used.

Action of Proteus rettgeri phage on other members of the family Enterobacteriaceae. The *P. rettgeri* phages did not have any action on any of the Salmonella, Shigella, paracolon or Pseudomonas strains tested. Phages isolated on *P. rettgeri* strains R65 and NCTC 7477 plated with efficiencies of 10^{-2} on two strains of *Escherichia coli*

(one strain locally isolated from faeces, the other a colicine F producing strain no. CA.42 supplied by Professor P. Fredericq, Liège, Belgium). The action of *P. rettgeri* phages on 24 different Providence strains is recorded in Table 4; 20 of the phages acted on one or more of these 19 strains. The e.o.p. varied between 10^{-4} and unity. A few systems with low e.o.p. were further examined. Enrichment of the top-layer agar with casein digest or with the electrolytes used before did not affect the e.o.p. Phage which had passed through a Providence strain plated with equal efficiency on this strain and the particular *P. rettgeri* host. One cycle of growth in this latter strain re-established the original plating efficiency. The cases examined appeared to be examples of phenotypic modifications of the particular phages.

Table 6. *Action of Proteus hauseri phage on P. morganii strains*

Plaque-forming titres were determined on homologous and heterologous hosts and relative efficiencies of plating calculated. Only systems showing one or more positive results are presented.

<i>P. hauseri</i> phages	<i>P. morganii</i>					
	NCTC 10041	NCTC 2815	M 235	M 232	M 47	M 21
6	-3	.	.	.	.	.
12	-3	A	.	A	A	.
14	-3	-3	.	.	.	A
19	-3	.	.	.	.	.
24	-3	A	.	.	.	A
34	-3	.	.	.	A	A
48	.	.	A	.	.	.
63	-3	A	.	.	.	.

Numbers = $\log_{10}$ of relative efficiency of plating. A = abortive infection. Points = no action.

Action of Proteus hauseri phages on P. rettgeri organisms. The e.o.p. of *P. hauseri* phages on strains of *P. rettgeri* is given in Table 5. The original observation by Vieu (1958) that some *P. hauseri* phages have an action on *P. rettgeri* strains was confirmed and extended. Eighteen of the *P. hauseri* phages caused productive infection in one or more of 15 *P. rettgeri* strains. The e.o.p. of most of the phages on the new hosts varied between 10^{-1} and 10^{-3} , but some plated with an efficiency of unity. Enrichment of the top-layer agar with amino acids or salts as before had no effect on the e.o.p. A few systems with low e.o.p. were further examined. A single passage through the heterologous host increased the e.o.p. to unity. The phenomenon was not further examined, it may be a phenotypic change like the cases previously mentioned. Discrepancies were sometimes observed in the host range of *P. hauseri* phages as determined by the spotting and titration techniques on *P. rettgeri* hosts. In these instances an area of bacterial inhibition in the form of complete or partial clearing was present where the drop of phage was placed but the plaque-forming titre of the phage on the particular organism was zero. These cases were all due to abortive infection with killing of the adsorbing organism. No phage production was ever found (Coetzee *et al.* 1960; Amati, 1962). As with efforts to increase the e.o.p. the top-layer agar was enriched with the electrolytes and amino acids in attempts to convert abortive infections to productive ones but no difference in the course of adsorption could be demonstrated.

Action of Proteus hauseri phage on P. morganii strains. Only organisms and phage which showed positive results are presented in Table 6. *P. hauseri* phage is decidedly less active on *P. morganii* strains than on *P. rettgeri* organisms. Only two *P. morganii* organisms (NCTC no. 10041, 2815) supported the productive cycle of one or more *P. hauseri* phages. The e.o.p. was 10^{-3} in all cases. The remaining positive reactions were all of the abortive infection variety associated with host killing. Vieu (1960) could not find any action by 33 temperate *P. morganii* phages on four strains of *P. hauseri*. Taubeneck (1962), with a series of temperate *P. hauseri* and *P. morganii* phages, did not find any action of *P. morganii* phage on *P. hauseri* strains; his *P. hauseri* phages also did not act on any *P. morganii* strains.

Action of Proteus hauseri phage on Providence strains. Only three strains showed evidence of phage action: strain NCTC 9219 supported productive infection by phages 19 and 74, strain NCTC 9224 was similarly attacked by phages 12 and 48, and strain NCTC 9246 was productively lysed by phages 12/57 vir, 6, 9, 12, 13, 14, 23, 24, 48, 51, 58, 67. The e.o.p. varied between 10^{-2} and unity.

DISCUSSION

Organisms able to support the growth of a particular phage are considered to be closely related (Stocker, 1955) and the many examples of productive infection caused by *Proteus hauseri* phages on *P. rettgeri* strains, and vice versa, should serve to strengthen the position of *P. rettgeri* within the *Proteus* group and confirms the findings of Falkow *et al.* (1962). The finding of Falkow *et al.* of very similar guanidine + cytosine contents in the deoxyribonucleic acids of Providence, *P. rettgeri* and *P. hauseri* strains may also reflect the finding that many *P. rettgeri* phages productively infect Providence organisms. The relationship between Providence and *P. hauseri* strains, on the other hand, is possibly less close as judged by the small number of Providence strains susceptible to *P. hauseri* phages.

The productive infection of a few *Proteus morganii* hosts by *P. hauseri* phage may also be regarded as evidence of a relation between these groups. That these interactions are not as frequent as those between *P. hauseri* and *P. rettgeri* strains and the fact that no *P. rettgeri* phage was found to attack *P. morganii* strains may support the finding of Falkow *et al.* (1962) that *P. morganii* strains are not as closely related to *P. hauseri* as are *P. rettgeri* organisms. The finding of two *P. rettgeri* phages which caused productive infection in 2 of 40 strains of *Escherichia coli* was interesting. At present these reactions appear to be rare; perhaps more weight should be given to the other reactions encountered.

The abortive infections encountered mean at the very least that the strains have common phage receptors. No abortive infections were encountered in the non-*Proteus* organisms tested and this adds to the significance of the finding.

The e.o.p. of phages on the heterologous hosts was often low but in all cases the phage progeny from the latter plated with the same efficiency on both the organisms involved. This investigation has shown close relationships between *P. hauseri*, *P. rettgeri*, Providence and to a lesser extent *P. morganii* strains which will have to be taken into account in any future taxonomic juggling with these organisms.

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REFERENCES

- ADAMS, M. H. (1959). *Bacteriophages*. New York: Interscience Publishers, Inc.
- AMATI, P. (1962). Abortive infection of *Pseudomonas aeruginosa* and *Serratia marcescens* with Coliphage P1. *J. Bact.* **83**, 433.
- BERTANI, G. & WEIGLE, J. J. (1953). Host controlled variation in bacterial viruses. *J. Bact.* **65**, 113.
- BRANDIS, H. & SCHWARZROCK, A. (1956). Über *Proteus*-Bakteriophagen. *Zbl. Bakt.* (I. Orig.), **165**, 226.
- COETZEE, J. N. (1958). The characterization of a series of *Proteus* bacteriophages. *S. Afr. J. lab. clin. Med.* **4**, 147.
- COETZEE, J. N., DE KLERK, H. C. & SACKS, T. G. (1960). Host range of lactobacillus bacteriophages. *Nature, Lond.* **187**, 348.
- COETZEE, J. N., PERNET, G. & THERON, J. J. (1962). Fimbriae and haemagglutinating properties in *Proteus* strains. *Nature, Lond.* **196**, 497.
- COETZEE, J. N. & SACKS, T. G. (1959). Lysogeny in the genus *Proteus*. *Nature, Lond.* **184**, 1340.
- COETZEE, J. N. & SACKS, T. G. (1960*a*). Lysogeny in the genus *Proteus*. 1. Incidence of lysogenic strains. *S. Afr. J. Lab. clin. Med.* **6**, 123.
- COETZEE, J. N. & SACKS, T. G. (1960*b*). Morphological variants of *Proteus hauseri*. *J. gen. Microbiol.* **23**, 209.
- COETZEE, J. N. & SACKS, T. G. (1960*c*). Transduction of Streptomycin resistance in *Proteus mirabilis*. *J. gen. Microbiol.* **23**, 445.
- DIENES, L. (1946). Reproductive processes in *Proteus* cultures. *Proc. Soc. exp. Biol., N.Y.* **63**, 265.
- EWING, W. H., TANNER, K. E. & DENNARD, D. A. (1954). The Providence group: an intermediate group of enteric bacteria. *J. infect. Dis.* **94**, 134.
- FALKOW, S., RYMAN, I. R. & WASHINGTON, O. (1962). Deoxyribonucleic acid base composition of *Proteus* and Providence organisms. *J. Bact.* **83**, 1318.
- KAUFFMANN, F. (1951). *Enterobacteriaceae*, 1st ed. Copenhagen: Munksgaard.
- KRIKLER, M. S. (1953). *The Serology of Proteus vulgaris*. Ph.D. Thesis: University of London.
- LURIA, S. E. (1953). Host-induced modifications of viruses. *Cold Spr. Harb. Symp. quant. Biol.* **18**, 237.
- NAMIOKA, S. & SAKAZAKI, R. (1959). New K antigen (C antigen) possessed by *Proteus* and *Reittergerella* cultures. *J. Bact.* **78**, 301.
- SHEDDEN, W. I. H. (1962). Fimbriae and haemagglutinating activity in strains of *Proteus hauseri*. *J. gen. Microbiol.* **28**, 1.
- STOCKER, B. A. D. (1955). Bacteriophage and bacterial classification. *J. gen. Microbiol.* **12**, 375.
- STORY, P. (1954). *Proteus* infections in hospital. *J. Path. Bact.* **68**, 55.
- TAUBENECK, U. (1962). Beobachtungen an lysogenen *Proteus*-stämmen. *Zbl. Bakt.* (I. Orig.), **185**, 416.
- Topley & Wilson's Principles of Bacteriology and Immunity* (1955). 4th ed. Ed. by G. S. Wilson and A. A. Miles. London: Edward Arnold.
- VIEU, J. F. (1958). Note préliminaire sur la lysotypie de *Proteus hauseri*. *Zbl. Bakt.* (I. Orig.), **171**, 612.
- VIEU, J. F. (1960). Lysogénité et sensibilité à la colicine H de *Proteus morgani*. *Ann. Inst. Pasteur*, **98**, 769.