Coupled mutations involving diverse characters in Salmonella Blegdam
by Leonard Raymond Bullas

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Genetics
Montana State University

Abstract:
Three independent derivatives of Salmonella blegdam, strain SB2, have exhibited the capacity to produce a temperate bacteriophage which lyses SB2. On seven occasions, lysogenic cells were isolated directly from these derivatives, The event leading to lysogeny or phage production occurs at mutational frequency. It was concluded, therefore, that SB2 carries at least most of the hereditary specifications of a temperate bacteriophage to which It is sensitive. Further mutational changes in phage related properties observed in SB2 derivatives included changes from sensitivity to immunity, changes from lysogeny back to sensitivity and changes from one level of lysogenic productivity and ultra-violet inducibility to another.

Most of the changes in phage related properties were accompanied by mutational changes affecting other unrelated bacterial properties which included nutritional requirements, motility, flagellar and somatic antigens, fermentation, colony morphology and antibiotic sensitivity. The prevalence of these coupled mutations argues that the changes in phage related properties are position effects rather than "point mutations".

Coupling between two or more mutations affecting non-phage related properties also occurs. Sometimes coupling is maintained for both forward and reverse mutation. This type of coupling usually involves a reciprocity, while one mutation restores a function, the coupled mutation abolishes another. In other situations, the coupling is lost once the mutations have occurred. Events occur which increase the probability of specific mutations. Some coupled mutations are "abortive" for they lead to functional genes which do not replicate. The cell has a "memory" for previous mutation so that reciprocal mutational changes occur between loci which were previously mutated.

Different auxotrophs showed different levels of UV inducibility. Examination of the degree of UV inducibility of particular auxotrophs led to the hypothesis that the site on the chromosome of a particular mutation was correlated with the location of prophage. Thus prophage is visualized as a movable element which can locate at many sites on the bacterial chromosome but is particularly 'attracted' to sites of mutation.

The hypothesis was advanced that the coupled mutations were controlled by an episomal element to which the symbol S was applied. This element can change position on the chromosome and suppress the activity and alter the mutability of adjacent genes. Prophage and S may occasionally become linked thus coupling bacterial mutations with phage property mutations.

COUPLED MUTATIONS INVOLVING DIVERSE CHARACTERS IN *SALMONELLA BLEGDAM*

by

LEONARD R. BULLAS

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

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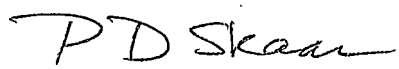
Genetics

Approved:

Head, Major Department



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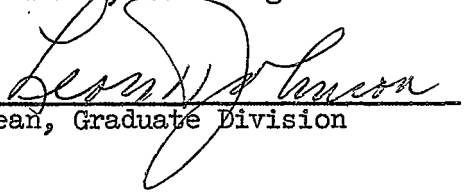

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ABSTRACT

Three independent derivatives of Salmonella blegdam, strain SB2, have exhibited the capacity to produce a temperate bacteriophage which lyses SB2. On seven occasions, lysogenic cells were isolated directly from these derivatives. The event leading to lysogeny or phage production occurs at mutational frequency. It was concluded, therefore, that SB2 carries at least most of the hereditary specifications of a temperate bacteriophage to which it is sensitive. Further mutational changes in phage related properties observed in SB2 derivatives included changes from sensitivity to immunity, changes from lysogeny back to sensitivity and changes from one level of lysogenic productivity and ultra-violet inducibility to another.

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GENERAL INTRODUCTION

The phylogenetic relationship between viruses and the genes of their hosts is of considerable theoretical and practical interest. Various bacteriophage-bacterium systems have proven extremely useful in approaching the problem. Within these systems a diversity of situations has been described, and these situations can be assigned (with varying degrees of certainty) to stages in the phylogeny of virulent phage from the normal bacterial genes (or *vice versa*). These include such phenomena as "weakly virulent" phage, virulent phage capable of lysogenization with the help of related temperate phage, classic inducible temperate phage, phage occupying specific sites on the bacterial linkage map, non-inducible temperate phage, defective temperate phage, host induced modifications, etc.

Until recently, however, no system has been available in which the entire sequence from sensitive to lysogenic bacterium could be studied (without the intervention of extra-clonal phage). This appears now to be provided by the *Escherichia coli* B - P2 system. The work of Bertani (1957) and Cohen (1959) had led to the suspicion that *E. coli* B carried genes capable of functioning in the temperate phage P2, and Rutberg and Hédin (1960) succeeded in provoking P2 release by pressurized sphaeroplasts of this strain. The preliminary report does not characterize the released phage in detail, but its identification as P2 implies a capacity to lysogenize *E. coli* B. Surprisingly, the transition from P2-sensitive to P2-releaser (and thence to P2-lysogenic by feasible population

interactions) is abrupt, and does not involve a long series of sequential events of mutational frequency.

A second system involving the entire sensitive-to-lysogenic transition has been discovered in Salmonella blegdam and is the subject of this report. Important differences from the E. coli B - P2 system have been revealed, but the general interpretation invokes, as does that of Rutberg and Hédin, profound spatial rearrangements within the cell as the basic cause of the transition.

In the course of the investigation, several other intimately related but independently significant phenomena were uncovered and are considered here. These include "shift mutations" from one auxotrophic state to another; reversible shifts from a monophasic (H antigen) condition to a diphasic condition involving a total of three antigens (two previously undescribed for S. blegdam); synchronized mutations of two or more such diverse genes as those determining immunity, phage production, auxotrophy, flagellar antigens, somatic antigens, sugar fermentation, colony morphology, flagellar production, flagellar function and antibiotic sensitivity; mutational events resulting in altered frequency of mutation; and mutant genes which show linear inheritance.

MATERIALS AND METHODS

A. Bacterial and Bacteriophage Strains

All bacteria employed in the present work were derived from two *Salmonella blegdam* strains originally studied at the University of Adelaide, Australia (Atkinson and Bullas, 1957). The following details about the origin of these strains are pertinent.

Through 1952 - 1957 a survey was conducted at the University of Adelaide to determine the incidence of lysogeny amongst different *Salmonella* groups. Many independent isolations from nature were studied, most of them from children and adults suffering from gastroenteritis. Among the strains studied were about fifty which were classified as *Salmonella blegdam*. That is, they possessed the somatic antigens 9 and 12 (thus belonging to Group D in the Kauffmann-White Schema) and were monophasic for the flagellar antigenic complex *gmq*.

The method used to determine lysogenicity was an adaptation of the cross-plating method developed by Fisk (1948) in which each culture is treated as both a potential lysogenic strain and as an indicator. By this method, only one *S. blegdam* strain (#43) could be shown to be lysogenic. With reference to the phage produced by #43, some of the remaining strains were resistant, some sensitive. One of the latter (#49) was selected as the standard propagating strain for future work. Since collections of most other *Salmonella* are predominately lysogenic (see Burnet 1932; Boyd 1950; Atkinson *et al* 1952), the demonstration of only one lysogenic *S. blegdam* was remarkable. The detection of lysogeny

is, of course, a function of the array of indicator strains available. Accordingly, it was suspected that the resistance of some of the S. blegdam strains to the phage produced by #43 might be due to cross-immunity conferred by an undetected prophage. In fact the unusual behaviour of #49 in serial broth culture suggested that it too might harbor a prophage or prophage-like element in a hitherto undescribed state, for such cultures rapidly and massively become resistant to the phage produced by #43. This suspicion is supported by the experiments reported here.

Strains #43 and #49 were selected for further study and here have been renamed SB1 and SB2 respectively. All other strains dealt with are derivatives of one of these two (namely SB2) and have been given designations such as SB3, SB4, SB5 etc. representing chronological order of isolation.

The phage produced by SB1 was originally called B43h/49 but has been renamed PSB1. PSB1 forms large turbid plaques on SB2 and is more completely described under Results. The phages produced by other bacterial strains are similarly named by inserting P before the name of the bacterial strain.

B. Culture Media, Diluents and Supplements

Except where indicated, all final concentrations given below are in grams per liter of distilled water. All culture media were sterilized in the autoclave at 15 lbs. pressure for 20 minutes.

Nutrient Agar (NA): Difco nutrient broth 8, NaCl 5, agar 15.

L-broth (LB): Bactotryptone 10, yeast extract 5, NaCl 5, glucose 1. The pH was adjusted to 7.2 with 1 N NaOH before sterilization. (Lennox, 1955)

L-agar (LA): L-broth ingredients 21, agar 10.

Top Layer Agar (TL): Difco nutrient broth 8, NaCl 5, agar 6.5.

Motility Medium (MM): Bacto Casitone 10, yeast extract 3, agar 4, Difco gelatin 80, NaCl 5. The pH was adjusted to 7.2 with 1 N NaOH after sterilization. (Edwards, 1942)

Eosin Methylene Blue Medium (EMB): Casein digest 10, yeast extract 1, NaCl 5, K_2HPO_4 2, Eosin Y 0.4, Methylene Blue 0.065, agar 15, sugar 10. (Lederberg, 1950)

Davis Minimal Liquid Medium (DMB): Glucose 1, K_2HPO_4 7, KH_2PO_4 2, crystalline sodium citrate 0.5, crystalline $MgSO_4$ 0.1, crystalline $(NH_4)_2SO_4$ 1; the sugar and the other salts were sterilized separately and added together only after sterilization. (Lederberg, 1950)

Nitrogen free DMB: Ingredients as for DMB with the omission of the $(NH_4)_2SO_4$.

Davis Minimal Agar (DMA): DMB ingredients 11.6, agar 15; the glucose and agar were added to a portion of the water, the other salts added to the remaining portion, each mixture sterilized separately and then added together.

Saline: NaCl 8.6.

Antibiotics: The following were added to media where appropriate to give the indicated final concentrations: Penicillin G, crystalline potassium (Eli Lilly and Co.) 300 µg/ml; Dihydrostreptomycin sulphate (Nutritional Biochemical Corp.) 250 µg/ml; Chloramphenicol (Pfizer Co.) 30 µg/ml.

Growth Factors: The following were added to minimal media where appropriate to give the indicated final concentrations, according to Lederberg (1950): Arginine .02 gm/ml, cytosine .02, glutamic acid .02, lysine .02, methionine .02, nicotinic acid .001, para-amino-benzoic acid .001, phenylalanine .02, tyrosine .02, tryptophan .02.

C. General Methods

1. Storage of Bacteria and Phage

Bacteria were stored on NA slants at room temperature, with routine subculture only at 12 monthly intervals. Strains in more general use were subcultured more frequently whenever it was deemed necessary.

Bacteriophage lysates were stored in the refrigerator at 4°C, after sterilization at 65°C - 70°C in a water bath, for 30 minutes.

2. Working Cultures

For most purposes, overnight (approx. 20 hrs.) L-broth cultures were employed. For certain purposes log phase cultures were obtained by inoculating 20 ml of L-broth in a Petri dish with 0.1 ml of an overnight culture and placing on a rotary shaker at 37°C for two hours.

3. Irradiation Procedures

Ultra-violet irradiation and induction experiments were carried out with a 2 bar ultra-violet lamp, model 2324, manufactured by the Dazor Manufacturing Corporation, St. Louis, Missouri, U.S.A. The lamp was placed at a distance of 50 cm from the center of a Petri dish containing 5 ml of the culture to be irradiated, on a rotating platform which rotated at a speed of approximately one revolution per second. Times of irradiation varied with the experiment but all irradiations were carried out with the same arrangement of lamp and culture.

4. Isolation of Mutants

The specific procedures used for the isolation of many mutants are detailed under the Results. The general methods employed for the isolation of auxotrophs were those of Lederberg (1950) and are given below.

$MnCl_2$ - Penicillin Method

SB2 was grown in L-broth at 37°C for 24 hours. The culture was then centrifuged and the sediment resuspended in 2 ml of 0.3 M NaCl and placed in a water bath at 37°C for one hour. The culture was again centrifuged, washed once in distilled water and resuspended in 2 ml of 0.04 M $MnCl_2$ solution for two hours at 37°C. After another centrifugation 1 loopful of the deposited cells, emulsified in the small amount of liquid that remained in the tube after decantation, was inoculated into 5 ml of L-broth and incubated overnight at 37°C.

The overnight culture was washed twice in sterile, distilled water and resuspended in 5 ml of nitrogen free DMB and incubated at 37°C for six hours. The culture was again centrifuged and resuspended in 5 ml of DMB with the addition of 300 units of penicillin-G. After overnight incubation, the culture was diluted out in saline and 0.1 ml of appropriate dilutions (10^{-4}, 10^{-5}, 10^{-6}) were plated out on NA to obtain isolated colonies. Plates showing suitable colony density were replicated to DMA (Lederberg and Lederberg, 1951) and incubated for 24 hours. The replicated DMA plates were then compared with the original NA plates and those colonies which appeared on the NA plates but not on the DMA plates were picked off and streaked on NA. A single colony was rechecked to test its auxotrophy, and its growth requirement identified.

Ultra-Violet Irradiation

NA plates spread with 0.1 ml of an undiluted overnight L-broth culture of SB2 were irradiated with UV for about 30 seconds and incubated overnight. The surviving colonies were then replicated to DMA and auxotrophic colonies identified in the same manner as used in the $MnCl_2$ - penicillin method.

5. Identification of Growth Factor Requirements

The method followed closely that described by Lederberg (1950). DMA plates were spread with 0.1 ml of appropriate growth factors and 1 loopful of the overnight L-broth culture, washed twice in saline, was streaked on the dried plate. The nature of the growth requirement was first identified by testing for growth with yeast extract and vitamin, amino acid and purine-pyrimidine pools. The individual components of the appropriate

pool which elicited growth were then tested singly and the specific growth factor identified.

6. Lysogenicity Tests

The method was adapted from that described by Fisk (1942) and is described here as the Mixed Growth Test. The potential lysogenic strain was grown in mixed culture on an L plate with SB2. After overnight incubation, the mixed growth was removed from the plate, emulsified in 1 ml. of L-broth and centrifuged to clarify. The clear supernatant fluid was then tested for lysis of SB2 on L-agar.

At the same time the culture was tested for sensitivity to lysis by phage PSB1 by the method of Craigie and Yen (1938) and described here as the Patch Test Method. This combination of two tests defines three types of culture:

(1) Cultures lysed by phage PSB1 but producing no phage in mixed culture with SB2 - called 'non-lysogenic';

(2) Cultures not lysed by phage PSB1 but producing no phage in mixed culture with SB2 called 'immune';

(3) Cultures not lysed by phage PSB1 and producing lysis of SB2 after growth in mixed culture - called 'lysogenic'.

Similar Mixed Growth Tests were performed using bacteria other than SB2 as potential propagating and indicator strain as described under Results.

7. Assays of Cells and Phage

Cells were assayed by diluting in saline and plating on NA.

Phage was assayed by the agar layer method originally introduced by

Gratia (1936), quoted by Adams (1959) and in general use by most phage workers. A two ml sample of melted TL medium at 45°C was inoculated with 0.2 ml of an overnight culture of indicator cells (SB2 unless otherwise stated); 0.1 ml of appropriate dilutions of phage in L-broth was added to each tube and the mixture poured onto the surface of a dried L-plate. After solidification, plates were incubated at 37°C overnight and the plaques counted.

Where desirable viable cells were destroyed by heating the lysate before assay at 65-70°C for 30 minutes.

8. Single Step Growth Curves of Phage

Single step growth curves of phages on SB2 were obtained following closely the methods outlined by Adams (1959). Three kinds of preliminary information were necessary: (1) The growth rate of SB2, (2) the rate of neutralization of the given phage by antiserum prepared against PSB1 and (3) the rate of adsorption of the given phage to SB2.

The growth rate of SB2 was determined as follows: Twenty ml of L-broth, inoculated with 0.1 ml of an overnight L-broth culture of SB2 was placed in a Petri dish on a rotary shaker at 37°C and the viable cells estimated by taking hourly samples. From the growth curve it was found that 1×10^8 viable bacteria/ml were obtained after about two hours incubation. Bacteria for the determination of neutralization rate and adsorption rate and the single step growth curve itself were grown under the same conditions as for the cell growth curve.

The rate of neutralization of phage by anti-PSB1 serum was determined

as follows: Antiserum was diluted 1/10 and 1/100 in L-broth. SB2, grown under standard conditions to approximately 1 x 10^8/ml was assayed. Phage was also diluted to approximately 1 x 10^8/ml and assayed. To 0.9 ml of the dilutions of antiserum was added 0.1 ml of the phage and the mixture placed at 37°C. At five and 10 minutes after mixing 0.1 ml samples were removed, added to 9.9 ml of chilled broth, and assayed for phage. The percentage of phage inactivated was calculated and the K value determined by the method of Adams (1959). The dilution of antiserum required to neutralize 99% of phage in five minutes was then calculated.

The rate of adsorption of a phage to SB2 was determined as follows: SB2 was grown under standard conditions to 1 x 10^8/ml and assayed. Phage was diluted to 1 x 10^8/ml and assayed. To 4.5 ml of culture at 37°C was added 0.5 ml of phage. At 10 and 15 minute intervals after mixing, 0.1 ml samples were removed and added to 9.9 ml of chilled L-broth. Volumes of 1 ml were centrifuged and assayed for phage. The percentage of phage adsorbed at 10 and 15 minutes was then calculated.

With the three preceding kinds of information single step growth curves were constructed as follows: SB2 was grown under standard conditions to 1 x 10^8/ml and assayed. Phage was diluted to 1 x 10^8/ml and also assayed. The phage antiserum was diluted out to the dilution calculated to neutralize 99% of phage in five minutes (1/4). The protocol of the single step growth curve was:

Time	Tube	Procedure
0 mins	1. adsorption	1. 0.1 ml phage added to 0.9 ml SB2.
5 mins	2. neutralization	2. 0.1 ml of 1. to 0.9 ml antiserum.
10 mins	3. dilution	3. 0.1 ml of 2. to 0.9 ml broth.
11 mins	4. F.G.T.	4. 0.1 ml of 3. to 9.9 ml broth.
12 mins	5. S.G.T.	5. 1.0 ml of 4. to 9.0 ml broth.

F.G.T. = First Growth Tube.

S.G.T. = Second Growth Tube.

All tubes were placed in a water bath at 37°C. At five minute intervals 0.1 ml samples were removed from the F.G.T. and S.G.T. and assayed for phage. From the results, the latent period and the average burst size were calculated.

9. Serological Methods

a. Antisera

Antisera against the Salmonella H antigens a, b, c, d, i and 1, 2, 3, 5, 6, 7 were obtained from Lederle. Other antisera were specifically prepared according to the following procedures.

(1) Vaccines

Methods in general use and described by Edwards and Ewing (1951) were applied. The H suspension consisted of an overnight L-broth culture of SB2 which had been inoculated from the edge of a swarm on MM. To the culture was added an equal volume of 0.6% formol-saline and the whole heated at 56° for 30 minutes.

O suspensions were prepared by removing the growth of SB2 from several

NA plates with saline, washing once in saline and placing the resulting suspension in a current of steam at 100°C for 2 1/2 hours. For injection, the suspension was diluted to an opacity equivalent to 10^9 organisms/ml.

Antiserum against phage PSB1 was made using a heat sterilized preparation of PSB1 with a titer of 5×10^8/ml.

(2) Production of Antiserum

Antisera were prepared in rabbits. Animals were bled from the marginal ear vein prior to the immunization and the resulting sera were first used as controls in agglutination or neutralization tests to detect any non-specific reaction.

Rabbits were injected intravenously into the marginal ear vein with 0.25 ml, 0.5 ml and thereafter 1.0 ml volumes of vaccine at intervals of three or four days. Injections were continued until a total of at least 10 ml of vaccine had been administered. Animals were bled on the fourth day after the last injection and if the antiserum so obtained was shown to have a high titer, the rabbits were bled again on the following second and fourth days. If more antisera were needed a second course of injections was commenced four to six months after the first course and the animals bled as before.

Antisera were stored in the freezing compartment of the refrigerator.

b. Agglutination Procedures

(1) H Agglutinations

Bacteria to be tested for H antigens were grown in L-broth overnight and an equal volume of 0.6% formol-saline added. The

suspensions were then heated at 56°C for 30 minutes to sterilize. Antisera were diluted in saline to appropriate concentrations. The Lederle antisera were used at the recommended 1/1,000 dilution. The dilutions found to be most useful for the SB2 H antiserum were 1/20, 1/1,000 and 1/10,000. Each agglutination tube contained 0.5 ml of diluted antiserum to which was added an equal volume of the antigen suspension. Tubes were placed in a water bath at 56°C for two hours and read for agglutination.

(2) O Agglutinations

Bacteria to be tested for O agglutination were grown on NA overnight. A thick suspension of the culture was made in 1 drop of saline on a slide. The O antiserum was diluted 1/5 in saline. One drop of the antiserum was mixed well with the bacterial suspension on the slide and the slide rotated to detect agglutination.

c. Phage Neutralization Tests

Antiserum was diluted 1/100 in L-broth. To 1.0 ml of diluted antiserum was added an equal volume of phage with a titer of about 10^7 phage particles/ml. The phage-antiserum mixtures were placed in a water bath at 45°C for 30 minutes and then assayed for unneutralized phage. The control consisted of a mixture of phage and L-broth which was similarly assayed. The percentage of phage neutralized under the given conditions was then estimated.

10. Cytological Methods

Bacteria examined to detect flagella were stained by the tannic

acid - basic fuchsin method of Leifson (1951). Bacteria emulsified carefully in distilled water after overnight growth on L-agar were found to give best results.

RESULTS

A. The Mutational Origin of Lysogeny

1. Capacity of SB2 to Give Rise to Lysogenic Cells

a. The Origin of SB5

With the object of isolating auxotrophic mutants, strain SB2 of Salmonella blegdam was subjected to $MnCl_2$ - penicillin screening. In one experiment, two different auxotrophs were obtained - SB4, a pyrimidine requiring strain satisfied by either cytosine or uracil, and SB5 which required lysine.

Immediately after isolation each strain was tested for sensitivity to phage PSB1. Since the parental strain SB2 is sensitive to this phage, the test constituted a convenient check against contamination. SB4 was lysed by PSB1 as expected but SB5 was not lysed. Since this result raised the possibility that SB5 was a contaminant, it was neglected for about three months. During this time it was stored on a NA slant at room temperature.

After three months of storage SB5 was tested to see if possibly it was lysogenic for a phage which, like PSB1, would lyse SB2. Using the sensitive Mixed Growth Technique (see Materials and Methods section) SB5 was grown with SB2 and the supernatant of the centrifuged mixed culture spotted on SB5 and SB2 in isolation. Lysis of SB2 but not SB5 was observed. Serial dilution of the supernatant confirmed that the lytic agent was a bacteriophage which formed hazy plaques indistinguishable from those of PSB1.

Serial streakings of SB5 on NA were then initiated. After 15 successive streakings, each originating from a well isolated colony on the preceding

plate, twenty colonies were picked and each subcultured to 5 ml L-broth. After overnight incubation the saturated cultures were tested for lysogenicity by the Mixed Growth Technique using SB2 as the second strain. All were positive. In addition, each culture was centrifuged and the supernatant subjected to a temperature of 65°-70°C to kill residual cells. The heated supernatants were then assayed for free phage by plating appropriate dilutions on SB2. Each contained free phage in concentrations varying from 160 to 4,000 per ml, with an average of 880.

Thus SB5 appeared to be a classic lysogenic strain in which the capacity to produce phage is an inherited property of all (or the vast majority) of the cells, and in which phage is released with a frequency which is low but still sufficiently high that free phage can be routinely detected in the medium in which it has been grown.

b. The Phage Released by SB5

The phages released by SB5 and SB1 were further studied. The plaque morphology of the former (hereafter called PSB5) was indistinguishable from that of PSB1. Both phages form plaques containing a central disk of resistant bacterial growth (sometimes centered with a small spot of lysis) surrounded by a clear ring of lysis. These plaques therefore resemble the plaque type of the temperate Type-A phages of S. typhimurium, photographs of which were published by Boyd (1950, 1954). Plaque morphology was the same regardless of whether the phages were plated on SB2 directly from the supernatant of the lysogenic or had been previously propagated on SB2. Lysates of both PSB1 and PSB5 regularly contained a small number of clear plaque

mutants. These phages formed plaques lacking the central disk of bacterial growth, but covered instead with a large number of resistant microcolonies that gave the plaques a "peppery" appearance when examined with a 10x lens.

Both PSB1 and PSB5 lyse SB2 but do not lyse either SB1 or SB5. Thus, by these limited criteria, their host ranges are identical.

Simultaneous phage neutralization tests employing antiserum prepared against PSB1 showed 92% neutralization of PSB1 and 90% neutralization of PSB5. Both phage preparations had been propagated on SB2. Thus PSB5 is serologically very similar to PSB1.

Atkinson and Bullas (1957) demonstrated that PSB1 (= phage B43h/49) was resistant to 80°C for 30 minutes. Simultaneous tests of PSB1 and PSB5, both propagated on SB2, showed identical responses. Thirty minute exposures to various temperatures showed no decrease in lytic activity up to 75°C, reduced lytic activity at 80°C and complete destruction of lytic activity at 85°C. Thus, in gross response of lysates to heating, the two phages were indistinguishable.

Six independent single step growth curves of PSB1 on SB2 showed latent periods of 30-35 minutes and burst sizes of 15 to 48 with an average of 31. Five independent single step growth curves of PSB5 on SB2 showed latent periods of 30 to 35 minutes and burst sizes of 31 to 61 with an average of 49. The single step growth curves for phage PSB1 and PSB5 are shown in figure 1. Insofar as these lytic cycle parameters are concerned, therefore, phages PSB1 and PSB5 are very similar. The difference in burst size may well be significant but the variation in values for each phage was too great