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Professor Joshua Lederberg,
Department of Genetics,
University of Wisconsin,
Madison 6, Wisconsin,
U.S.A.

Dear Professor Lederberg,

Many thanks for your kind letter of January 8th. I was not sure if I was on your mailing list and I am very happy to know that I am. There is no hurry for the reprints, send them at your own convenience. I shall of course send you mine as they appear.

Professor Rubbo has now finalized the lecture schedule for the year and you have no doubt received all the details. As you can see on the schedule, you will be doing all the genetics lectures during the second term, and I do a few odd ones in the third term. I give about 10 lectures on bacteriophage in the first term and will include phage genetics and the inheritance of prophage.

Regarding your suggestion on running through some experiments together while you are here, I am most happy at this suggestion, and I wondered if it might be worth while to consider this aspect in greater detail now. I understand from Professor Rubbo that he had nothing definitely planned for your wife to do, and I would like to present for your approval a proposal that she work with me on the inheritance of lysogenicity in Pseudomonas. Professor Rubbo is quite happy about this suggestion and it is up to you to say whether you are interested.

I have had in mind for some time to work on this subject but pressure of other things resulted in it being shelved until about five months ago when I was presented (by my organism) with such an unusual phage system that I felt I should be flying in the face of Fate to neglect it. During some other work I put up the cross $1(T_1^-)$ x $L(IV^-)SC$ (nomenclature of my 1955 paper) as a comparison of recombination frequency. This cross usually has a R.F. of about 100 but on this occasion the R.F. was only about 5. The stock of $L(IV^-)SC$ was one which had been subcultured a month or so before and kept at $-4^{\circ}C$. It had not been used for several months. To cut a long story short, this clone of $L(IV^-)SC$ has become spontaneously lysogenic for at least 4 phages, two

of which do not act on L strains. This particular clone I have called L(IV$^-$)SCLys.

It occurs to me at this point to say that this whole investigation is still very much in the preliminary state and certainly not suitable yet for publication. I found the stock about last October and very soon afterwards went on my annual leave. I managed to get 4-5 weeks work on it before the lab shut down for 3 weeks at Xmas, and since Xmas I have been working under some difficulties as the rest of the Department has shifted to the new Medical School building and I have remained in the old buildings (with only limited facilties). The last few weeks have been very unsettled and I have done little work. This letter is being written during the physical and mental trauma of packing. I give this in apologia, as further work may reveal different conclusions to those which I am giving you now.

I have temporarily called these phages LP, LD, LS and LX. LP, LD and LS act on strain 1 but LX only on Strain L. All these phages are clearly distinguishable on plaque morphology, serology, or host range, or a combination of these characteristics. I have not done much at all with LS or LX but have concentrated mainly on LP and LD. These latter phages are unrelated, so it is possible to make the double and single lysogenic derivatives of strain 1. I have isolated a virulent mutant of LD (called LDV) and another virulent phage, LV, acting on both bacterial strains L and 1. LV is unrelated to LP and LD. The nomenclature is lousy I know and at the moment is nothing more than laboratory slang. It is possible to get phage resistant mutants of strain 1 to both virulent strains LDV and LV. Those resistant to LDV are also resistant to LD; those resistant to LV are resistant to LP, LD, and LDV.

I have carried out some crosses to study the inheritance of prophage, without I'm afraid entirely conclusive results to date. My technique for crossing is to mix doubly washed suspensions of each parent, plate immediately onto minimal, and wait three days, then with a moistened loop, touch a recombinant colony and then streak the loop over the surface of a minimal plate. After overnight incubation a single colony is picked from this plate and subcultured into liquid minimal tubes. After 24 hours growth this culture is then spotted onto a plate flooded (and subsequently dried) with the phage indicator strain.

I have found that if the cross is set up so that the acceptor parent (strain 1) is susceptible to the phage carried by the donor parent then spurious ratios of lysogenic and non lysogenic progeny are obtained. For this reason, the non lysogenic parent must be made resistant to external infection by the lysogenic phage carried by the other parent. As strain L is not susceptible to the LP or LD phages I thought it best to arrange things so that both parents would be susceptible to the phages. I did this by crossing 1($M_1^-T_1^-$) x L($IV_1^-Ad^-$) on minimal plus isoleucine,

valine and tryptophane and selecting for IV^-T^- recombinants of such mating type that they would cross to strain 1. Such recombinants (or the dozen or so I tested) were found to have the same reactions to the group of phages as strain 1. The one strain I used I call R (for recombinant) 629 (IV^-T^-). I carried out the following crosses -

$$1(LIL^-)SC\ LDV^r \times R629(IV^-T^-)(LD)^+$$

i.e. strain 1 parent, requiring leucine or isoleucine, resistant to streptomycin and chloramphenicol, resistant to virulent phage LDV, non lysogenic for LD, crossed to recombinant strain 629 requiring isoleucine + valine, and tryptophane, streptomycin and chloramphenicol sensitive and lysogenic for phage LD.

On testing the recombinants for lysogenesis 1 recombinant out of 180 was lysogenic for LD, S^r and C^r and resistant to LDV. Segregation for S and C was normal. Recombination frequency was lower but not significantly so from normal. This one recombinant remained stable through a second single colony isolation.

A second cross was carried out with the parents.

$$1(LIL^-)SC\ LV^r \times R629(IV^-T^-)(LD)^+(LP)^+$$

7 recombinants were found to be LV^r and lysogenic, 3 lysogenic for LD, 3 lysogenic for LP and 1 lysogenic for both. After a second single colony isolation only one recombinant was lysogenic for LP, and one for LD. This instability of lysogenesis in recombinants has been noticed with repeat crosses. Whether it represents a characteristic of the phage, a characteristic of prophage inheritance or merely a technical difficulty is not known at present.

Two other crosses were made -

(1) $1(T_1^-)LV^r \times L(IV_1^-)SC$ Lys. Of 143 recombinants, 33 were lysogenic for LD, 3 for LP and 3 for both. 22 were, however, lysogenic for what is thought to be LS but this has not been checked carefully and of these 22 some were possibly lysogenic for either LP or LD but this could not be ascertained for sure. 5 were lysogenic for a phage not identical with either LP, LD or LS. Lysogenesis was determined using strain 1 as the susceptible indicator strain.

(2) $1(LIL^-)LV^r \times L(IV_1^-)SC$ Lys. Of 92 recombinants 21 were lysogenic for LD, none for L, none for both, 13 for LS, 7 for the unidentified phage.

When the LD and LP lysogenic recombinants from these 2 cases were single colonied only 1 recombinant was still lysogenic (for LD) and it was resistant to LV.

The cross $1(LIL^-)(LD)^+(LP)^+$ x $L(IV_1^-)SC$ Lys. had a recombinant frequency of about 10 (normal about 200) suggesting that the main cause of the loss of recombinants is not LP or LD but possibly LS or the other phage and when I get set up in Melbourne I will get on to this straight away.

Certainly I am convinced that the LP and LD prophages have been transferred at zygote formation although at a very low frequency.

There is an additional fact for which I have no explanation and unless it can be shown that it is a technical artifact may prove to be a little disconcerting. This is the fact that stocks of phage LP always contain phage LDV, whether prepared by the agar plate method, induction or broth lysis. Single plaques of LP have been taken, dispersed in broth and in about 10-20% found to contain LDV (10^{-1}-10^{-2} per plaque). My immediate reaction to this observation was contamination but with additional work this view is becoming increasingly difficult to maintain. In actual fact in spite of the fact that I am working with all these phages contamination has been very rare. I have even tried making stocks of LP in other labs where no LDV has ever been but I still get it under these circumstances.

This is a rather complicated story not very well told and I only hope that you can get some idea of the picture. Would you like me to send you the relevant bacterial and phage strains before you come to Melbourne? I look forward to hearing your comments on this whole proposal. I might add that should you decide you would like to work on this topic I would be most delighted.

I think it would be a very good idea for you to bring out your fluorescens strains, indeed you may like to send them out beforehand. I think this may be an idea anyway, you may have customs difficulties if you are actually carrying cultures. It might be an idea to discuss this with Professor Rubbo, he would know the regulations in greater detail than I do.

With my best wishes,

Yours sincerely,

Bruce Holloway

B.W.Holloway.

P.S. I am leaving for Melbourne in a day or so, therefore when you reply do so to the Department of Bacteriology University of Melbourne.

BH