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Dr. A. Gourevitch
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Dear Alex:

I am delighted to see how well the induction procedure has been sharpened up. With a 10^3- or higher-fold increase in yield of plaques with 1 ug/ml of azaserine, you should certainly be in a position to screen for comparable agents. There is no reason you should not proceed with the technique you have worked out.

The reason I originally suggested using 10^8 cells was to allow the dilution step that you (for other reasons) prefer to avoid. My reasoning was as follows. Induction is a two-stage process: 1) the "activation" of the prophage [whatever this means] and 2) the growth of the phage and lysis of the treated cells. Step 1 can be conducted under a variety of conditions: e.g., with UV even in buffer suspension, but 2) requires that the cells be optimally situated for synthetic processes. I was afraid that you might run into some broths in which the antibiotic itself, or some other constituent, would have so much antibacterial activity that step 2) would be inhibited. I thought that one should separate the activation step 1) by dilution so that step 2) would occur in a noninhibitory broth. This is only a premonition, and no antibiotic might actually work out that way. You will have to decide yourself whether the risk of losing such effects would be worth the added effort of a dilution step.

On the other hand, this problem suggests the possibility of looking for still another kind of antibiotic activity, namely for agents that will interfere with step 2) of phage growth. The procedure would be to use cells that had been induced previously with UV (or if you prefer azaserine) and test your broths for the ability to inhibit the expected production of lambda. Streptomycin, e.g., is known to have this effect (I don't know of any antibiotic, in fact, that does not). I realize that you should not go off in all directions in a screening program, but if you have developed your procedure for this kind of test, you might want to think about it. It would be of considerable interest, I think, to identify 1) inhibitors of bacteria that do not prevent induction, and 2) probably more exciting, non-bacterial-inhibitors that do prevent phage growth. In addition, the inhibition test would be another check in looking for the more ordinary kinds of antibacterial substances. Let me know if you or Joe wants to discuss this possibility any further, and I won't elaborate any further now. I assume that your present screening with K-12 is done independently of whether you detect antibacterial activity of the broths.

It is too bad you have to nurse your cultures at home. Why not set up a clock-control that runs cold water through a small thermostated-bath until a given time, then stops the water and turns on the heat? With an electric interval timer, a solenoid valve, and a relay, it's easily done. Alternatively

older cultures which are diluted 1:5 or 1:10 and regrown should be ready within a couple of hours.

The lamda should be diluted either in a balanced salts solution, or preferably ordinary Difco nutrient broth, rather than the buffer. Even distilled water would probably give you reasonably clean results. I can't see why plate replications should vary— are these inoculated simultaneously from the same pipette?

Weigle says a small dose of UV (say 5 secs of a sterilamp [15Watt]) at 50 cm) given to the sensitive bacteria before they are plated evens out the plaque morphology considerably, if such a complication would be worth the trouble to you.

Sincerely,

Joshua Lederberg